SHOUT
SHARE
IT

PROCEEDINGS

OF THE

ROYAL SOCIETY OF LONDON

Series B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

VOL. LXXVI.

LONDON:
Printed for THE ROYAL SOCIETY and Sold by
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HIS MAJESTY.

NOVEMBER, 1905.

SMITHSONIAN
LIBRARY

LONDON:
HARRISON AND SONS, PRINTERS IN ORDINARY TO HIS MAJESTY,
ST. MARTIN'S LANE.

SMITHSONIAN
LIBRARY

CONTENTS.

SERIES B. VOL. LXXVI.

No. B 507.—April 22, 1905.

PAGE

Address delivered by the President, Sir William Huggins, K.C.B., O.M., F.R.S., at the Anniversary Meeting on November 30, 1904 1

Researches on some of the Physiological Processes of Green Leaves, with special Reference to the Interchange of Energy between the Leaf and its Surroundings. By Horace T. Brown, LL.D., F.R.S., and F. Escombe. (This paper, and the three following ones, constituted the Bakerian Lecture for 1905.) 29

On a New Method for the Determination of Atmospheric Carbon Dioxide, based on the Rate of its Absorption by a Free Surface of a Solution of Caustic Alkali. By Horace T. Brown, F.R.S., and F. Escombe 112

On the Variations in the Amount of Carbon Dioxide in the Air of Kew during the years 1898–1901. By Horace T. Brown, F.R.S., and F. Escombe 118

On the Thermal Emissivity of a Green Leaf in Still and Moving Air. By Horace T. Brown, LL.D., F.R.S., and W. E. Wilson, D.Sc., F.R.S. 122

No. B 508.—May 24, 1905.

On the Absence or Marked Diminution of Free Hydrochloric Acid in the Gastric Contents, in Malignant Disease of Organs other than the Stomach. By Benjamin Moore, M.A., D.Sc., Johnston Professor of Bio-Chemistry in the University of Liverpool (in collaboration with W. Alexander, M.D., M.Ch., F.R.C.S., Hon. Surgeon, Royal Southern Hospital, Liverpool; R. E. Kelly, M.B., B.Ch., B.Sc., Alexander Fellow, University of Liverpool; and H. E. Roaf, M.B., Johnston Colonial Fellow, University of Liverpool). Communicated by Professor C. S. Sherrington, F.R.S. 138

On Reciprocal Innervation of Antagonistic Muscles.—Seventh Note. By C. S. Sherrington, F.R.S. 160

A Preliminary Note upon the Question of the Nutrition of the Early Embryo, with Special Reference to the Guinea-pig and Man. By E. Emrys-Roberts, M.B. (Liverpool), Ethel Boyce Research Fellow in Gynæcological Pathology in the University of Liverpool. Communicated by Professor C. S. Sherrington, F.R.S. 164

The Influence of Cobra-venom on the Proteid Metabolism. By James Scott, M.D., C.M., B.Sc. Edin. Communicated by Sir Thomas R. Fraser, F.R.S. 166

On the Physical Chemistry of the Toxin-Antitoxin Reaction: with Special Reference to the Neutralisation of Lysin by Antilysin. By J. A. Craw,

PAGE

Research Student, Lister Institute of Preventive Medicine. Communicated by Dr. C. J. Martin, F.R.S. 179

Two Cases of Trichromic Vision. By F. W. Edridge-Green, M.D., F.R.C.S. Communicated by Dr. F. W. Mott, F.R.S. 194

The Colour-Physiology of the Higher Crustacea, Part III. By Frederick Keeble, M.A., University College, Reading, and F. W. Gamble, D.Sc., Zoological Department, Victoria University, Manchester. Communicated by Professor Sydney J. Hickson, F.R.S. (Abstract) 198

On Colour-Vision by Very Weak Light. By George J. Burch, M.A., D.Sc. Oxon.; F.R.S.; Lecturer in Physics, University College, Reading 199

No. B 509.—June 28, 1905.

On the Nature of the Silver Reaction in Animal and Vegetable Tissues. By A. B. Macallum, M.A., M.B., Ph.D., Professor of Physiology in the University of Toronto. Communicated by W. D. Halliburton, F.R.S. 217

On the Resemblances existing between the "Plimmer's Bodies" of Malignant Growths, and Certain Normal Constituents of Reproductive Cells of Animals. By J. Bretland Farmer, F.R.S., J. E. S. Moore, and C. E. Walker 230

Observations on the Brains of Men and Animals infected with Various Forms of Trypanosomes. Preliminary Note. By F. W. Mott, M.D., F.R.S. 235

On a New Rhabdosphere. By George Murray, F.R.S. 243

On some New Species of Lagenostoma, a Type of Pteridospermous Seed from the Coal Measures. By E. A. Newell Arber, M.A., F.L.S., F.G.S., Trinity College, Cambridge, University Demonstrator in Palæobotany. Communicated by D. H. Scott, F.R.S. (Plates 1 and 2) 245

Ovulation and Degeneration of Ova in the Rabbit. By Walter Heape, M.A., Trinity College, Cambridge. Communicated by Adam Sedgwick, F.R.S. 260

On Reciprocal Innervation of Antagonistic Muscles.—Eighth Note. By C. S. Sherrington, D.Sc., F.R.S. 269

No. B 510.—August 3, 1905.

On the Dimorphism of the English Species of *Nummulites*, and the Size of the Megalosphere in Relation to that of the Microspheric and Megalospheric Tests in this Genus. By J. J. Lister, M.A., F.R.S., Fellow of St. John's College and Demonstrator of Comparative Anatomy in the University of Cambridge. (Plates 3—5) 298

On the Occurrence of *Anopheles* (*Myzomyia*) *Listoni* in Calcutta. By J. R. Adie, M.B. (Lond.), Major, I.M.S., and A. Alcock, M.B., LL.D., F.R.S., Major, I.M.S. 319

The Structure and Function of Nerve-Fibres.—Preliminary Communication. By J. S. Macdonald, Professor of Physiology in the University College, Sheffield. Communicated by Professor C. S. Sherrington, F.R.S. 322

On the Effect of Carbon Dioxide on Geotropic Curvature of the Roots of *Pisum sativum*, L. By Eric Drabble, D.Sc., F.L.S. (Demonstrator in Botany, Royal

PAGE

College of Science), and Hilda Lake. Communicated by J. Bretland Farmer, F.R.S. 351

Preliminary Note on the Occurrence of Microsporangia in Organic Connection with the Foliage of Lyginodendron. By R. Kidston, F.R.S.L. and E., F.G.S. (Plate 6) 358

On the Efferent Relationship of the Optic Thalamus and Deiter's Nucleus to the Spinal Cord, with Special Reference to the Cerebellar Influx Theory (Hughlings Jackson) and the Genesis of Decerebrate Rigidity (Sherrington). By F. H. Thiele, M.D., B.Sc., M.R.C.P., Pathologist to University College Hospital. Communicated by Sir Victor Horsley, F.R.S. 360

On Endophytic Adaptation shown by *Erysiphe Graminis* DC. under Cultural Conditions. By Ernest S. Salmon, F.L.S. Communicated by Professor H. Marshall Ward, F.R.S. (Abstract) 366

A Preliminary Communication on the Life History of *Trypanosoma Balbianii*. By W. S. Perrin, B.A., Shuttleworth Research Student of Gonville and Caius College, Cambridge. Communicated by A. Sedgwick, F.R.S. 368

On the Chemical Mechanism of Gastric Secretion. By J. S. Edkins, M.A., M.B. Cantab., Lecturer on Physiology in the Medical School of St. Bartholomew's Hospital, London. Communicated by Professor C. S. Sherrington, F.R.S. 376

A Preliminary Note on the Susceptibility of Goats to Malta Fever. By Dr. T. Zammit, Member of the Mediterranean Fever Commission. Communicated by Colonel D. Bruce, C.B., F.R.S., R.A.M.C. 377

Preliminary Note on Goats as a Means of Propagation of Mediterranean Fever. By Major W. H. Horrocks, R.A.M.C., Member of the Mediterranean Fever Commission. Communicated by Colonel Bruce, C.B., F.R.S. 378

No. B 511.—September 2, 1905.

On the Occurrence of Certain Ciliated Infusoria within the Eggs of a Rotifer, considered from the Point of View of Heterogenesis. By H. Charlton Bastian, M.A., M.D., F.R.S. (Abstract.) (Plate 7) 385

The Morphology of the Ungulate Placenta, particularly the Development of that Organ in the Sheep, and Notes upon the Placenta of the Elephant and Hyrax. By Richard Assheton, M.A., Lecturer on Biology in the Medical School of Guy's Hospital, University of London. Communicated by A. Sedgwick, F.R.S. (Abstract) 393

Contributions to the Physiology of Mammalian Reproduction. Part I.—The Œstrous Cycle in the Dog. Part II.—The Ovary as an Organ of Internal Secretion. By Francis H. A. Marshall, Carnegie Fellow in the University of Edinburgh; and William A. Jolly, Assistant to the Professor of Physiology in the University of Edinburgh. Communicated by Professor E. A. Schäfer, F.R.S. (Abstract) 395

Chitin in the Carapace of *Pterygotus osiliensis*, from the Silurian Rocks of Oesel. By Otto Rosenheim, Ph.D. Communicated by Professor W. D. Halliburton, F.R.S. 398

PAGE

On a New Species of *Cephalodiscus* (*C. nigrescens*) from the Antarctic Ocean. By E. Ray Lankester, M.A., D.Sc., LL.D., F.R.S., Director of the Natural History Departments of the British Museum. (Plate 8) 400

Experimental Researches in Vegetable Assimilation and Respiration. IV.—A Quantitative Study of Carbon-Dioxide Assimilation and Leaf-Temperature in Natural Illumination. By F. Frost Blackman, D.Sc., Fellow of St. John's College, Reader in Botany in the University of Cambridge, and Gabrielle L. C. Matthaei, B.A., Fellow of Newnham College. Communicated by Francis Darwin, For. Sec. R.S. 402

Note on the Mechanics of the Ascent of Sap in Trees. By Professor J. Larmor, Sec. R.S. 460

No. B 512.—October 7, 1905.

On the Cytology of Apogamy and Apospory.—II. Preliminary Note on Apospory. By Miss L. Digby. Communicated by Professor J. B. Farmer, F.R.S. 463

The Pharmacology of Indaconitine and Bikhaconitine. By J. Theodore Cash, M.D., F.R.S., and Wyndham R. Dunstan, M.A., LL.D., F.R.S. 468

The Synthesis of a Substance allied to Adrenalin. By H. D. Dakin. Communicated by Professor E. H. Starling, F.R.S. 491

On the Physiological Activity of Substances Indirectly Related to Adrenalin. By H. D. Dakin. Communicated by Professor E. H. Starling, F.R.S. 498

Transmission and Inoculability of *Spirillum Theileri* (Laveran). By Dr. A. Theiler. Communicated by Colonel David Bruce, R.A.M.C., F.R.S. 504

An Experimental Enquiry into the Nature of the Substance in Serum which influences Phagocytosis. By George Dean, M.A., C.M., M.B., Bacteriologist-in-Charge of the Serum Department of the Lister Institute of Preventive Medicine, London. Communicated by Professor J. Rose Bradford, F.R.S. 506

The Phagocytosis of Red Blood-Cells. By J. O. Wakelin Barratt, M.D., B.Sc. Lond., British Medical Association Research Student. Communicated by Sir Victor Horsley, F.R.S. 524

Report on the Anatomy of the Tsetse-fly (*Glossina palpalis*). By E. A. Minchin, M.A., Professor of Zoology and Comparative Anatomy in University College, London, late Fellow of Merton College, Oxford. Communicated by Professor E. Ray Lankester, F.R.S. 531

No. B 513.—November 9, 1905.

The Dual Force of the Dividing Cell. Part I.—The Achromatic Spindle Figure Illustrated by Magnetic Chains of Force. By Marcus Hartog, M.A., D.Sc., F.R.U.I. Communicated by Sir W. Thiselton Dyer, F.R.S. (Plates 9—11) ... 548

On the Probable Existence of Emulsin in Yeast. By Thomas Anderson Henry, D.Sc. (Lond)., Principal Assistant in the Scientific and Technical Department of the Imperial Institute, and S. J. M. Auld, Ph.D. (Würzburg), Assistant in the Scientific and Technical Department of the Imperial Institute. Communicated by Professor Wyndham Dunstan, F.R.S. 568

PAGE

On the Inheritance of Heterostylism in *Primula*. By W. Bateson, M.A., F.R.S., and R. P. Gregory, M.A. 581

Further Experiments and Histological Investigations on Intumescences, with some Observations on Nuclear Division in Pathological Tissues. By Miss Elizabeth Dale. Communicated by Professor H. Marshall Ward, F.R.S. (Abstract)...... 587

The Experimental Treatment of Trypanosomiasis in Animals. By H. Wolferstan Thomas, M.D., C.M. (McGill) (J. H. Todd Memorial Fellow in Tropical Medicine), Liverpool School of Tropical Medicine, Johnston Tropical Laboratory, University, Liverpool. Communicated by Professor R. Boyce, F.R.S. ... 589

Studies on Enzyme Action. VII.—The Synthetic Action of Acids contrasted with that of Enzymes. Synthesis of Maltose and Isomaltose. By Edward Frankland Armstrong, Ph.D., D.Sc., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E. Armstrong, F.R.S. 592

Studies on Enzyme Action. VIII.—The Mechanism of Fermentation. By Edward Frankland Armstrong, Ph.D., D.Sc., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E. Armstrong, F.R.S 600

Studies on Enzyme Action.—Lipase. By Henry E. Armstrong, F.R.S. 606

Index 609

PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Address delivered by the President, Sir William Huggins, K.C.B., O.M., F.R.S., at the Anniversary Meeting on November 30th, 1904.

Since the last Anniversary the Society has lost by death fourteen Fellows. The deceased Fellows are:—

Sir Frederick Bramwell, born 1818, died Nov. 30, 1903.
Robert Etheridge, born Dec. 3, 1819, died Dec. 18, 1903.
George Salmon, born Sept. 25, 1819, died Jan. 22, 1904.
Lieut.-General C. A. McMahon, born March 23, 1830, died Feb. 21, 1904.
Sir C. Le Neve Foster, born Mar. 23, 1841, died April 19, 1904.
George Johnston Allman, born 1824, died May 8, 1904.
Alexander William Williamson, born May 7, 1824, died May 6, 1904.
Robert McLachlan, born April 10, 1837, died May 23, 1904.
Isaac Roberts, born 1829, died July 17, 1904.
Sir John Simon, born Oct. 10, 1816, died July 23, 1904.
Joseph David Everett, born 1831, died Aug. 9, 1904.
Sir William Vernon Harcourt, born Oct. 14, 1827, died Oct. 1, 1904.
Frank McClean, born 1837, died Nov. 8, 1904.
Earl of Northbrook, born 1826, died Nov. 15, 1904.

Memorial Notices of the Fellows who have been taken from us by death during the past year will appear in due course in the Obituary Notices.

Of some of them only, on this occasion, will time permit me to give expression, on your behalf, to a few words of appreciation of their work, and of deep sorrow at their loss.

In your name I place a wreath, emblem of our respect and of our deep sorrow, to the memory of our late Fellow and Copley-Medallist, the revered Provost of Trinity College, Dublin, who passed away at the ripe age of eighty-four years. George Salmon was as remarkable in the influence of his powerful personality, as in his works, by which he extended and adorned two domains of thought, as diverse as mathematics and theology. It is given to few men to achieve a European reputation as an investigator of the first rank in two distinct provinces of knowledge.

Born and educated in the City of Cork, he matriculated at Trinity College, Dublin, at the early age of fourteen. After a brilliant undergraduate course, he took his degree in 1838, and was elected a Fellow in 1841. Devoting himself to the study of pure mathematics, he produced a series of books, now accounted as classics in every university of the world, which were of very great service in promoting the advancement of that science. Their value was shown by the number of their editions, by their translation into several languages, and by the honours they procured for their author. In his "Lessons Introductory to the Study of the Modern Higher Algebra," which grew in subsequent editions until it became a treatise, he made accessible to the student the recent researches of the previous twenty years into the theory of transformations of binary forms.

Following the traditions of the Dublin School of Mathematics, he gave wide scope in all his books to geometrical method, often relieving the monotony of pages of analysis by the introduction of a brilliant geometrical proof.

In 1866, on the preferment of Dr. Butcher, Salmon was appointed Regius Professor of Divinity, from which time he ceased to work at mathematics, except in an occasional way at the Theory of Numbers. This is not the place for a consideration of his contributions to theological literature, nor of his great influence in the Church in Ireland at a time of exceptional difficulty. One important aspect of his theological labours is expressed by the title which was given to him of "*malleus Germanorum.*"

In the year 1888 he was appointed by the Lord Lieutenant to the post of Provost of Trinity College. His large sympathy with all sorts and conditions of men, his unaffected dignity, his genial humour, and his kind heart, gave to his masterful tenure of the office of Provost an influence probably unparalleled in the history of Trinity College.

Not Trinity College alone, but all Dublin was proud of him. Men of all classes and creeds praised him. His private tastes were simple; his chief relaxations, chess playing, music, and novel reading. In the words of the late Bishop of Oxford:—"The Provost is an extraordinary man. The first day I met him I was most struck by his gracious courtesy, the second day by his learning, the third day by his humour, and every day by his humility."

The Fates are inexorable; there may be long delay, but always at last the thread is cut. In midsummer our oldest Fellow, in point of election as well as of age, passed from us:—Sir John Simon, the pioneer of modern sanitary science. What Lister did for surgery, and Pasteur for bacteriology, Simon may be said to have accomplished for sanitation. Very early he perceived clearly and developed the true nature and mode of dealing with contagious emanations proceeding from the sick, establishing a doctrine and practice which afterwards received their direct proof and further development in the growth of the new science of bacteriology. Deeply grateful to his memory, we mourn one who by his life-work conferred incalculable benefit upon the whole civilized world.

Simon commenced the study of medicine in 1833, and attended both St. Thomas's Hospital and the recently established King's College. It was in 1848 that his attention was definitely directed to that branch of the profession with which his name will always remain famous, and which indeed he may almost be said to have founded, through his election to the newly-constituted post of Medical Officer of Health to the City of London. Seven years later a Central Board of Health was created, on which Simon represented medicine. When the functions of the Board were transferred to the Privy Council, he became adviser to the Government on all sanitary and medical matters. It is not possible on this occasion to indicate, even broadly, his strenuous work through a long life for the public good. His writings consist mainly of his numerous official reports, together with a volume published in 1857, entitled "Papers on the History and Practice of Vaccination," followed in the next year by a "Report on the Sanitary State of the People of England," which brought out for the first time the wide variations which exist in the local incidence of diseases. His great work on "English Sanitary Institutions" appeared in 1890. In 1878 he was elected President of the Royal College of Surgeons; he was the recipient of numerous honours from scientific bodies at home and abroad. At the Jubilee in 1887 he received from Queen Victoria the distinction of K.C.B. These public recognitions were the outward signs of the universal respect and honour accorded him by all men. His memory will ever remain green in the history of sanitary science.

In May passed away, full of years and full of honours, a Fellow to whose personal services the Society is largely indebted—Professor Williamson. Elected into the Society in 1855, after serving twice upon the Council, he became Foreign Secretary in 1873, which office he held for sixteen years, until 1889. Half a century ago Williamson took a prominent part in the development of chemical thought, and exercised a powerful influence on chemical teaching in this country. He began the study of chemistry at Heidelberg, but soon passed to Liebig's laboratory at Giessen, where he took his degree, and while there published papers on the decomposition of Oxides and Salts by Chlorine, and on "The Blue Compounds of Cyanogen and Iron." He then went to Paris, where he came under the teaching of Comte. In 1849 he left Paris to occupy the chair of practical chemistry in University College, from which he continued to teach for thirty-eight years. A little later he published the classical research, elucidating the process of the formation of ether, with which his name will always remain associated. This paper, a model of concise reasoning founded upon happily devised experiment, produced a profound influence on contemporary thought, and received the assent of the whole chemical world. In this paper he gave his acceptance of the doctrine of types, which was prominent in his subsequent teaching. Williamson was a pioneer of chemical thought in quite another direction by the introduction of the conception of dynamics into chemical processes. He advanced the view, which is fundamental in the modern hypothesis of ionic dissociation, that in substances which appear at rest, the atoms of the molecules of the compound are in motion, exchanging from one molecule to another in an unending course of ionic migrations.

Williamson occupied the chair of the British Association in 1873, and was twice President of the Chemical Society. Honorary degrees were conferred upon him by the Universities of Dublin, Edinburgh, and Durham, and he received the honorary membership of many scientific societies. Seventeen years ago he retired from professional life to Hindhead.

Alas ! this room will know no more a frequent and welcome attendant at our meetings who often took part in our discussions. A man whose great natural vitality and intellectual activity were so remarkable and unimpaired, that his sudden death came as a great shock to his many friends. Professor Everett was born and educated at Ipswich, and after graduating with honours at Glasgow, he became Professor of Mathematics at King's College, Nova Scotia. Later, in 1867, he was appointed Professor of Natural Philosophy at Queen's College, Belfast, a chair which he occupied with distinction for thirty years. Since his retirement, about seven years ago, he has resided in London, taking an active part in the proceedings of scientific societies, especially of the Physical Society, of which he was a Vice-President. Professor Everett rendered important service to physical science, by his admirable translation of Deschanel's "Treatise of Natural Philosophy," which he brought up to date from time to time by the necessary additions and alterations, and by his "Illustrations of the C.G.S. System," which was translated into several languages, and proved of material service in the establishment of a physical system of units. He did important work as the secretary of the Committee of the British Association which effected the selection and naming of these dynamical and electrical units, and also of the Committee which has collected our main knowledge of underground temperatures. He was the inventor of a system of shorthand, which provides greater facilities for vowel insertion than other systems. He was enthusiastically devoted to cycling. A man of great kindliness and geniality, he is regretted by a large circle of friends, and will always be remembered by his numerous pupils with much gratitude and affection.

Death has deprived us of a Fellow whose genial humour, clear judgment, and ready wit endeared him to many friends—Sir Frederick Bramwell. In Bramwell the love of things mechanical was inborn. At the time of his youth, technical education was all but unknown, and very few engineering students could take advantage of such a meagre scientific education as was then available. He was a striking example of what he himself said of some distinguished engineers :—"That they literally became such because they could not help it." With Bramwell the taste for engineering was innate and supreme. Study was not congenial to him; his extensive and varied knowledge was mainly the outcome of personal observation and experience.

After some years' varied experience in different engineering workshops he commenced practice on his own account in 1853. He soon made his mark; but, as he especially shone in debate, where his judgment was rarely at fault, and he brought shrewd common sense to bear with happy flashes of wit and apt practical illustrations, he was irresistibly drawn from the constructive to the legal side of his profession, in which he received no little advantage from his powerful voice and his commanding presence. In giving evidence, Bramwell was remarkably able, and as an arbitrator his judgments were clear, judicial, and marked by legal acumen. In one or other capacity his services were in much demand during the last thirty or forty years. He was chosen President of the Institution of Mechanical Engineers in 1874, and, ten years later, President of the Institution of Civil Engineers. He was President of the British Association at its meeting at Bath in 1888. He became one of our Fellows in 1873, and served on the Council in 1877-1878. On the retirement of Sir William Bowman, he was elected Honorary Secretary of the Royal Institution. Honorary degrees were conferred upon him by the Universities of Oxford, Cambridge, Durham, and Montreal. In 1889 Queen Victoria bestowed upon him the honour of a baronetcy.

George Johnston Allman was born in Dublin in 1824. He entered Trinity College at an early age, and at the honour degree examination, in 1843, he obtained Senior Moderatorship and a gold medal in mathematics. A few years later he was elected to the Professorship of Mathematics in Queen's College, Galway, a post which he held for nearly forty years, until his retirement in accordance with the age limit. His most important works were a paper, "On some Properties of the Paraboloids," and a series of papers on the history of Greek mathematics, which formed the basis of his celebrated book "Greek Geometry from Thales to Euclid." He was elected a Fellow of the Society in 1884.

The name of Dr. Isaac Roberts will always be associated with the photography of the heavenly bodies. He early showed his love for physical science. His first scientific paper was on the wells and water of Liverpool, where he resided; and in the following year, 1870, he was elected a Fellow of the Geological Society. Other papers followed on underground waters, especially with respect to their oscillations in porous strata. He soon directed his principal attention to Astronomy, and erected an observatory near Liverpool. At first he contemplated photographing the whole northern heavens, but when an astrographic chart and catalogue for both hemispheres were undertaken by an international co-operation of Observatories, with great prescience he decided to devote himself to photographing star-clusters and nebulæ. Finding the neighbourhood of Liverpool unfavourable for such work, after a long personal examination of various sites, he erected an observatory on Crowborough Hill, where, during thirteen years, he secured the splendid series of astronomical photographs, bringing to light a wealth of unsuspected detail, which have largely aided in the recent extension of our knowledge of nebulæ and star-clusters. Two volumes containing reproductions of these photographs were published by Dr. Roberts at his own expense, and widely distributed among astronomers. He was elected to our Fellowship in 1890. In 1892 Trinity College, Dublin, conferred upon him the honorary degree of D.Sc.; three years later he received the gold medal of the Royal Astronomical Society.

To his many friends the sudden death of Sir Clement le Neve Foster came as a very painful shock. He was educated in France, and obtained the degree of Bachelor of Science of the University of France at the early age of sixteen. He then entered the Royal School of Mines, where in two years he achieved the remarkable distinction of securing the Associateship in the Mining, Metallurgical, and Geological divisions, as well as the Duke of Cornwall's Scholarship and the Forbes Medal. In 1872 he was appointed H.M. Inspector of Mines. He succeeded, in 1890, Sir Warrington Smyth as Professor of Mining at the Royal College of Science, and the Royal School of Mines. He became a Fellow of our Society in 1892. On the King's birthday, last year, he received the honour of Knighthood. During his twenty-nine years' Government Inspectorship, Sir Clement did much to ameliorate the lot of the miner, and to establish metal mining on a scientific basis.

Quite recently the Society has suffered a further loss in the unexpected death of Dr. McClean, who, by his wisely considered benefactions, as well as by his personal work, has contributed not a little to the increase of natural knowledge. Having retired thirty-four years ago from professional work as an engineer, he built an astronomical observatory at his house at Tunbridge Wells, and devoted himself to photo-spectroscopic work on the sun and stars. His photographic spectra of all stars above the $3\frac{1}{2}$ magnitude appeared in our *Transactions*, in which he showed the presence of oxygen in connection with helium in certain stars. His benefactions to Science are of two kinds. In 1890 he founded the Isaac Newton Studentships at Cambridge for the promotion of the study of Astronomy and Astronomical Physics; while, on the practical side, ten years later, he made a most generous gift of valuable instruments to the Royal Observatory at the Cape of Good Hope. He has crossed the great bar, to the deep sorrow of his many friends, and to the great regret of all men of science.

During the last few years a very large amount, increasing each year, of work outside the reading, discussion, and printing of papers, of a more or less public character, has been thrown upon the Royal Society—so large indeed as at present to tax the Society's powers to the utmost. A not inconsiderable part of this work has come from the initiation by the Society itself of new undertakings, but mainly it has consisted of assistance freely given, at their request, to different Departments of the Government on questions which require expert scientific knowledge, and which involves no small amount of labour on the part of the Officers and Staff, and much free sacrifice of time and energy from Fellows, in most cases living at a distance.

There is little doubt that this largely-increased amount of public work has arisen, in part naturally from the greater scientific activity of the present day, but also, and to a greater extent, from the fuller recognition by the Government and the public of the need for scientific advice and direction in connection with many matters of national concern.

It may not be inopportune, therefore, for me to say a few words on the advisory relation in which the Society has come to stand to the Government, and to review very briefly the great work which the Society has done, and is doing, for the Nation.

Among Academies and Learned Societies the position of the Royal Society is, in some respects, an exceptional one. In the British dominions it holds a unique position, not only as the earliest chartered scientific Society, but in its own right, on account of the number of eminent men included in its Fellowship, and the close connection in which it stands, though remaining a private institution, with the Government. The Royal Society is a private learned body, consisting of a voluntary and independent association of students of Science united for the promotion of Natural Knowledge at their own cost. It asks for no endowment from the State, for it could not tolerate the control from without which follows the acceptance of public money, nor permit of that interference with its internal affairs which, as is seen in some foreign academies, is associated with State endowment. In one particular case, in which it can receive aid without any loss of independence, the Society gratefully acknowledges its indebtedness to the State. About 1780 the Society received a communication from the Government offering to provide apartments for the Society at Somerset House; these were exchanged, in 1857, for rooms in old Burlington House; after its rebuilding, in 1873, the Society moved into the apartments which it now occupies. It should not be forgotten that nearly a century before the opening of the British Museum in 1759, the Royal Society's Museum, or Repository as it was called, enjoyed the prestige of being regarded as the most important Museum in London, and must have been of great use to men of science, and have aided materially in promoting and disseminating the knowledge of natural history. The apartments offered to the Society at Somerset House were quite insufficient in capacity and in number to receive the Society's Museum, and in consequence, this collection, which had been carefully maintained not only from the scientific side, but also with reference to the commercial value and importance of the foreign objects received, especially of the valuable zoological specimens frequently sent by the Hudson's Bay Company from their territories, was presented by the Society to the Nation, a not unworthy acknowledgment, on the Society's part, of the Government's gift of apartments. This collection has not been kept separate, but is now hopelessly dispersed among the thousands of specimens which crowd the halls of our National Museum. Some specimens, however, in comparative anatomy, preserved in the Museum of the College of Surgeons, are duly entered in the catalogue as having belonged originally to the Royal Society's Museum.

Besides the grant of apartments in Somerset House, and subsequently in Burlington House, the Society has received no pecuniary support from Government, nor assistance of any kind, with one exception to be mentioned further on, beyond the grant by Charles II. shortly after its incorporation, of Chelsea College and the lands appertaining to it; a gift which proved much less valuable than appeared from the parchments. Claimants at once came forward for portions of the estate, and the property was in so unsettled a state as to title, and so much out of repair, that after much money had been spent on repairing the College and great exertions made in vain to procure a tenant, the President was authorised to sell the estate to the King for the sum of £1,300; the Council voting their thanks to him for "thus disposing of a property which was a source of continual annoyance and trouble to them." To the extent of this sum the Society's funds were enriched by the royal gift.

The grants of £4,000 and £1,000 now received annually by the Royal Society from the Government are not applicable to its own needs, but are placed in its hands in trust for grants in aid of the prosecution of scientific research, and of the publication of scientific papers; indeed, with the exception of part of the publication grant, are so far from being of the nature of a State bounty, that the careful administration of these grants brings no light burden upon the Society.

It may not be generally known that the Royal Society just missed becoming a richly-endowed Society. Charles II.'s interest in the young Society did not end with the grant of a Charter of Incorporation, for in 1662 he addressed a letter, written with his own hand, to the Duke of Ormonde, then Lord Lieutenant of Ireland, recommending the Royal Society for a "liberal contribution from the adventurers and officers of Ireland for the better encouragement of them in their designs." That is to say, in the new settlement in that country, on the Restoration, of the confiscated estates of such persons as by the King's declaration were disqualified. The Royal Society had but a poor chance, notwithstanding the King's letter, of coming in for a portion of these so-called "fractions," when so many high families were cheated of their rights, and the Duke's own estates, through his methods of adjudication, increased from £7,000 to £80,000 per annum. Sir

William Petty, in a document preserved in the archives of the Society, estimates the value of the lands granted by the King to the Society, but not received by them, "as a great matter, but I know not what."

It is on record that the non-fulfilment of the King's generous intentions towards the Society did not damp the philosophic ardour of the Fellows; indeed, it is a question on which opinions may widely differ whether the rich endowment of the Society, almost from its very birth, would have increased its scientific success. We must not forget that, in the case of institutions as well as of individuals, the powerful and healthy stimulus to the exertion needful for success which arises from the necessity of coping with and overcoming difficulties, whether of a monetary or other kind. In no small degree was due to the personal favour with which Charles II. regarded the Society, the exceptional position it early took up, and which it still holds to-day, of a private institution supported and controlled from within, which, at the same time, is acknowledged by the State as the authoritative national representative of Science in this country, and from time to time consulted as such.

The first royal act which distinctly gave this representative character to the newly chartered Society appears to have been the King's declaring his pleasure on the 15th October, 1662, "that no patent should pass for any philosophical or mechanical invention until examined by the Society." This personal recognition by the King of the national position of the Society was followed and confirmed a few years later by a request from the department of the Admiralty for assistance from the Royal Society in raising some ships sunk off Woolwich. The Council replied that, though they would have great pleasure in affording all assistance in their power by advice, the want of funds rendered it impossible for them to provide the necessary machinery.

From that time down to the present the Royal Society, while remaining a purely private institution for the promotion of Natural Knowledge, has been regarded by the Government as the acknowledged national scientific body, whose advice is of the highest authority on all scientific questions, and the more to be trusted on account of the Society's financial independence; a body, which, through its intimate relations with the learned societies of the Colonies, has now become the centre of British Science. The Society's historical position and the scientific eminence of its Fellows have made it naturally the body which the scientific authorities of foreign countries regard as representing the Science of the Empire, and with which they are anxious to consult and to co-operate, from time to time, on scientific questions of international importance.

On their part, the Fellows of the Royal Society, remembering that the promotion of Natural Knowledge is the great object for which it was founded and still exists, and that all undertakings in the home and in the State, since they are concerned with Nature, can be wisely directed and carried on with the highest efficiency only as they are based upon a knowledge of Nature, have always recognised the fundamental importance of the Society's work to national as well as to individual success and prosperity, and their own responsibility as the depositories of such knowledge. They have always been willing, even at great personal cost, ungrudgingly to afford any assistance in their power to the Government on all questions referred to them which depend upon technical knowledge, or which require the employment of scientific methods. In particular the Society has naturally always been eager to help forward, and even to initiate, such national undertakings as voyages of observation or of discovery of any kind, or for the investigation of the incidence of disease, which have for their express object the increase of Natural Knowledge.

At the same time, as the Society is dependent upon the voluntary help of its Fellows, whose time is fully occupied with their own work, the Society may reasonably expect the Government not to ask for assistance on any matters of mere administration that could be otherwise efficiently provided for. The hope may be expressed that in the near future, with increased official provision in connection with the recognition of Science, the relation of the Society to the Government may not extend beyond that of a purely advisory body, so that the heavy responsibilities now resting upon it, in respect of the carrying out of many public undertakings on which its advice has been asked, may no longer press unduly, as they certainly do at present, upon the time and energy of the Officers and Members of Committees. The Society regards this outside work, important as it is, as extraneous, and therefore as subordinate, and would not be justified in permitting such work to interfere with the strict prosecution of pure natural science as the primary purpose of the Society's existence, upon which, indeed, the Society's importance as an advisory body ultimately depends.

The array of national undertakings of which the Society has been wholly or in part in charge, or to which it has given advice or assistance from time to time, is so very great that any attempt to point out, even in broad outline, the more important of the directions in which the Society's influence has been actively employed for the public service, must necessarily be fragmentary and very incomplete. On this occasion it is not possible to do more than to give, in a few sentences, a rapid presentation of a few typical examples of the Society's public work.

It must be borne in mind that the bare statement in a few sentences of the public work accomplished by the Society fails altogether to bring before the imagination an adequate conception of the large amount of free labour ungrudgingly given by those Fellows who composed the several committees to which the work was entrusted.

Going back to the first century of the Society's existence, the work done for the National Observatory at Greenwich may be fairly taken as typical of the Society's outside activity at that time. It is not too much to say that the Observatory owes, in no small degree, its early efficiency and the high position it soon reached, to the advice and the energetic action on its behalf of the Royal Society. The Observatory, at the time it was placed, in 1710, by Queen Anne in the sole charge of the Society, was without instruments, except such as Flamsteed had himself supplied. Immediately on taking charge, the Society appointed a Committee which visited Greenwich, and, as a result, sent in an application to the Ordnance Office, but at the time unsuccessfully, for the new instruments which were absolutely essential for properly carrying on the work of an observatory. The little interest taken by the Government of that day in Science is manifest from the answer received from the Ordnance Office, "that they had never been at any charge for instruments, but only for repairing the house and paying Mr. Flamsteed's salary." The Society persevered, and when, in 1720, Halley succeeded Flamsteed, was successful in persuading the Government to provide a few of the more necessary instruments. At a little later date the Society induced the Government to expend £1,000 on instruments, to be constructed by Graham and Bird. When George III. came to the throne he re-appointed the Society as sole visitors, and ordered the Astronomer Royal to obey the regulations drawn up by the Council, and commanded the Master General of Ordnance to furnish such instruments as the Council should think necessary for the Observatory. In the list of these instruments is mentioned a ten-foot telescope of Dollond's "new invention." Further, it was in answer to a petition from the Royal Society that the King gave orders for the printing of the Observations made at the Observatory. At a later date the Society called on the Government to advance funds to establish magnetical observatories at Greenwich, and in various parts of the British dominions, with the result that in a few years no fewer than forty magnetical establishments were in full activity.

In connection with the Observatory may be mentioned the considerable share which the Society took in bringing about the important alteration of the Calendar, known as the Change of Style, which took place in 1752. The

Bill was drawn up by Peter Davall, the Secretary of the Society, aided and supported by Lord Macclesfield, who became President the same year. The change was approved and assisted by the actual President, Martin Folkes. The feeling of the people was so strongly against the change that the illness and death of Bradley, who as Astronomer Royal had assisted the Government with his advice, which took place not long afterwards, were popularly attributed to a judgment from Heaven.

Very brief must be the mention of some of the other works in the public service which were carried out at a no small cost of labour to the Fellows of the Society.

About 1750, the Lord Mayor of London, two of the Judges and an Alderman, having died in one year from jail-fever caught at the Old Bailey Sessions, the Society was called upon for advice and assistance. A committee was appointed to investigate the wretched state of ventilation in jails. A ventilator, invented by one of the committee, was erected in Newgate, reducing at once the number of deaths from eight a week to about two a month. Of the eleven workmen employed to put up the ventilator, seven caught the fever and died.

At the request of the Government, committees were appointed to consider the best form of protection of buildings, and, later on, of ships at sea, from lightning.

The Society took a very active part in the measurement of a degree of latitude, afterwards in the length of a pendulum vibrating seconds in the latitude of London, and in the comparison of the British Standards with the Linear Measure adopted in France. A committee was appointed to compare the Society's Standard yard with that of the Exchequer. Later, in 1834, when the Standard yard was lost in the destruction by fire of the Houses of Parliament, a Commission (all the members of which were Fellows of the Royal Society) was appointed to consider the steps to be taken for the restoration of the Standards.

It was at the instance of the Council of the Society, who petitioned George III. for the necessary funds, that the King gave his consent to a geodetical survey in 1784, with the immediate object of establishing a trigonometrical connection between the Observatories of Greenwich and Paris. The work, under General Roy, for which the Copley Medal was awarded to him, served as a basis for the operations of a more extensive nature, embracing a survey of the British Islands, which were commenced in 1791.

Since its foundation the Society has taken an active part in many important expeditions for scientific and geographical exploration, and for magnetical and astronomical observations, in some cases taking the initiative by memoralizing the Government for the necessary assistance by grants of money, the use of ships, or otherwise. Among these may be mentioned the expeditions sent out for the observation of the Transits of Venus in 1761, and in 1769.

The importance of Antarctic exploration, for which the recent National Expedition has recently been promoted jointly with the Royal Geographical Society, was fully understood by the Royal Society nearly a century and a half ago. In 1771, an expedition having for its principal object the exploring of high southern latitudes with the view of ascertaining the existence of a great Antarctic Continent, was strongly and successfully urged on the Government by the Society. The expedition under Captain Cook sailed the following year. On its return three years later, after having circumnavigated the globe, the Copley Medal was awarded to Captain Cook for the means he had taken to preserve the health of his crew.

In 1817, a letter was addressed by Sir Joseph Banks, on the part of the Council, to Lord Melville urging that an expedition of discovery should be sent out for determining the practicability of a North-West Passage. The Lords of the Admiralty gave orders for the fitting out of four vessels, and invited detailed instructions from the Royal Society for the guidance of the officers. The Council recommended Colonel, then Captain, Sabine to proceed with the North-West Expedition, and Mr. Fisher to accompany the Polar one. The expedition failed to procure geographical results of importance, but it was far from fruitless, for the magnetical observations brought back by Sabine were an addition of real value to physical science.

This expedition was followed by another two years later under Parry, which resulted in the discovery of the Strait called after Barrow, then Secretary to the Admiralty.

A later Polar Expedition, under Captains Parry and Ross in 1827, was promoted by the Royal Society, and brought home valuable magnetical observations, which were printed in the Society's Transactions.

At home, it was through the Society's influence that Dr. Maskelyne, the Astronomer Royal, was able to make observations in Scotland for the purpose of deducing the density of the earth. Dr. Hutton undertook the laborious task of working up the data, the whole expenses being borne by the Society.

These few examples, inadequate as they are, must suffice on this occasion to remind us of the many labours during two centuries and a half undertaken by the Society for the public good. I pass now at once to some of the many objects of public concern, which are at the present time either directly promoted, or assisted by the Society.

The establishment in this country of a National Physical Laboratory for the purpose of bringing scientific knowledge to bear practically upon the industries and commerce of the nation, was due in no small measure to the action of the Society, and has certainly thrown upon it much additional permanent responsibility. The necessity for such an Institution in this country, which was clearly shown by the marked influence of a similar Institution on the improvement of technical science and the manufacturing interests of Germany, had been already strongly advocated by individual Fellows; in particular, by Sir Oliver Lodge at Cardiff in 1891, and Sir Douglas Galton at Ipswich five years later; but the first practical step towards its realisation was taken by the Council in 1896, when they decided that the Royal Society should join the British Association and other kindred Societies in a Joint Committee, under the Chairmanship of the President of the Royal Society, to take such action as they find desirable.

In the following year, this Committee waited upon Lord Salisbury, who was then Prime Minister, and, as a result, a Treasury Committee was appointed by the Chancellor of the Exchequer, with Lord Rayleigh as Chairman, to consider the desirability of establishing a National Laboratory. That Committee, after hearing witnesses and visiting Germany, reported strongly and unanimously in favour of such a national Institution. In 1898, a communication was received from the Treasury expressing "the hope that the Royal Society will be willing to add to the already great services rendered by them to the Government and public of the United Kingdom, by consenting to undertake the new responsibilities now sought to be imposed upon them" in connection with the new Institution. The Council accepted the important trust, under which the "ultimate control of the Institution is vested in the President and Council of the Royal Society, who in the exercise thereof may issue from time to time such directions as they may think fit to the General Board and Executive Committee." The income and all other property is vested in the Royal Society for the purposes of the Institution. The Laboratory, which was formally opened by H.R.H. the Prince of Wales in March, 1902, has already made remarkable progress under its energetic Director. During the present year the attention of the Prime Minister has been called to the very great importance to the national industries of an immediate grant for new buildings and a more adequate instrumental equipment, and of a larger annual endowment.

It is not too much to say that men of Science of all countries are under no small obligation to the Royal Society for their Catalogue of Scientific Papers which have appeared in all parts of the world since the beginning of the last century. This great work, to which immense labour has been given gratuitously and without stint by Fellows during the past forty years, will be carried down to the close of the century, and will consist of two parts: an Authors' Catalogue, and a Catalogue of Subjects. Encouraged by a donation from Mr. Andrew Carnegie, and the noble liberality of Dr. Ludwig Mond and other Fellows, the Council decided to proceed with the completion of the Catalogue, in the hope of further donations from Fellows and others as the work advances.

It was obvious that to continue permanently to prepare and publish catalogues of the rapidly increasing output of scientific literature would be wholly beyond the means of any one Society, and was an undertaking so vast as to require organized international co-operation for success. In 1893, a letter, signed by seventeen Fellows, was addressed to the President, asking that steps might be taken to provide for the continuation of the Society's Catalogue from the beginning of the century by adequate international co-operation. A Committee was appointed, which reported in favour of an international conference on the subject. Three conferences were held successively in 1896, 1898, and 1900. It is scarcely possible to convey an adequate conception of the arduous and prolonged labours of these conferences, and of the numerous meetings of committees held in connection with them. The Society may well feel great satisfaction that a work of such magnitude, and of so great moment to all scientific workers, which was initiated by itself, was taken up with such remarkable accord by the scientific world. The organization consists mainly of a Central Bureau in London under the Royal Society, in connection with Regional Bureaus, established in thirty countries for collecting material in the form of catalogue slips, and transmitting them to the Central Bureau. The Royal Society has taken upon itself practically the financial responsibility of the undertaking, making contracts in its own name with a printer and a publisher, the latter undertaking the technical duties as agent for the Society, which is its own publisher. The first year's issue of the catalogue has appeared, dealing in twenty-one volumes with the seventeen sciences decided upon by the conference.

The International Association of Academies, the realization for the first time of the great scientific idea of a Universal Academy, open without restriction of language or of country to every nation under heaven, owes its establishment to the initiative of the Royal Society. In 1897, the Royal Society was invited to send representatives to a Conference of a Union of German Academies and Societies which met from time to time. The Society sent delegates, but declared that the Society's permanent adhesion to any such association must be conditional on its being made truly international in character. The principle of an international association of learned Societies suggested by the Royal Society, was accepted, and a Conference was held at Wiesbaden in 1899 for the purpose of taking steps for the formation of such an association. Statutes were drawn up and arrangements made for the holding of the first General Assembly in Paris in 1901.

The primary objects of the Association are the initiation and promotion of scientific undertakings of general interest and of universal concern to mankind, especially of such matters as are outside the power of a single Academy and require for their promotion the assistance of the Governments represented by the Association. Indirectly by its triennial General Assemblies in different countries, it should become an instrument of no mean power for the promotion of the brotherhood of mankind and for hastening the day

> "When the war drums throb no longer and the battle flags are furl'd,
> In the Parliament of man, the Federation of the world."

The Association, as now constituted, consists of twenty Academies and learned Societies of Europe and America. The second General Assembly of the Association was held this year in London under the auspices of the Royal Society, which, as directing Academy, had had general charge of the conduct of its business during the last three years. The Section of Letters met under the direction of the newly-founded British Academy.

The Society has accepted heavy responsibilities at the instance of the Government in respect of the control of scientific observations and research in our vast Indian Empire. In 1899, the India Office inquired whether the Royal Society would be willing to meet the wishes of the Indian Government by exercising a general control over the scientific researches which it might be thought desirable to institute in that country. A Standing Committee was appointed in consequence by the Council for the purpose of giving advice on matters connected with scientific enquiry, probably mainly biological, in India, which should be supplementary to the Standing Observatories Committee which was already established at the request of the Government as an advisory body on astronomical, solar, magnetic, and meteorological observations in that part of the Empire.

An investigation, onerous indeed, but of the highest scientific interest and of very great practical importance, has been carried on by a series of Committees successively appointed at the request of the Government for the consideration of some of the strangely mysterious and deadly diseases of tropical countries. In 1896 a Committee was appointed at the request of the Colonial Secretary to investigate the subject of the Tsetse Fly disease in South Africa. Two years later Mr. Chamberlain, Secretary of State for the Colonies, requested the Society to appoint a Committee to make a thorough investigation into the origin, the transmission, and the possible preventives and remedies of tropical diseases, and especially of the malarial and "Blackwater" fevers prevalent in Africa, promising assistance, both on the part of the Colonial Office and of the Colonies concerned. A Committee was appointed, and, under its auspices, skilled investigators were sent out to Africa and to India. In the case of the third Committee the Society itself took the initiative. An outbreak in Uganda of the disease, appalling in its inexorable deadliness, known as "Sleeping Sickness" having been brought to the knowledge of the Society, a deputation waited upon Lord Lansdowne at the Foreign Office, asking him to consider favourably the despatch of a small Commission to Uganda to investigate the disease. He gave his approval, and a Commission of three experts, appointed on the recommendation of the Committee, was sent out to Uganda, £600 being voted out of the Government Grant towards the expenses of the Commission.

The investigations in tropical diseases, promoted and directed by these Committees, have largely increased our knowledge of the true nature of these diseases, and, what is of the highest practical importance, they have shown that their propagation depends upon conditions which it is in the power of man so far to modify, or guard against, as to afford a reasonable expectation that it may be possible for Europeans to live and carry on their work in parts of the earth where hitherto the sacrifice of health, and even of life, has been fearfully great. A general summary of the work already done on Malaria, especially in regard to its prevention, and also on the nature of "Blackwater" Fever, has been published in a Parliamentary paper, which records Mr. Chamberlain's acknowledgment to the Royal Society for its co-operation in the work undertaken by the Colonial Office. Our Reports on Sleeping Sickness up to this time form four parts of a separate publication giving evidence in support of the view that this deadly disease is caused by the entrance into the blood, and thence into the cerebro-spinal fluid, of a species of Trypanosoma, and that these organisms are transmitted from the sick to the healthy by a kind of tsetse fly, and by it alone; Sleeping Sickness is in short a human tsetse fly disease.

In 1897, the Council was requested to assist the Board of Trade in drawing up Schedules for the establishment of the relations between the Metric and the Imperial Units of Weights and Measures. A Committee was appointed, which, after devoting much time and attention to the matter, drew up Schedules which were accepted by the Board of Trade and incorporated in the Orders of Council.

A Coral Reef Committee has been in active existence for some years, and has directed the attempts to pierce, by boring, the atoll of Funafuti, towards the expenses of which grants have been made by the Council. The results of the work have appeared in a large volume, giving a description of the whole core from the points of view of the naturalist and the chemist; and a list, with critical remarks, of the species of animals and plants collected.

Soon after the reports were received of the appalling volcanic eruptions and the loss of life which took place in the West Indies in 1902, the Council received a letter from Mr. Chamberlain to ask if the Society would be willing to undertake an investigation of the phenomena connected with the eruptions. The Council, considering that such an investigation fell well within the scope of the objects of the Society, organized a small Commission of two experts, who left England for the scene of the eruption eleven days only after the receipt of Mr. Chamberlain's letter; the expenses being met by a grant of £300 from the Government Grant Committee. Six weeks were spent in the Islands, including Martinique, by the Commission, which was successful in securing results of great scientific interest. A preliminary report was published at the time, and a full report has since appeared in the "Transactions."

Time forbids me to do more than mention the sucessive expeditions sent out by the Society, conjointly with the Royal Astronomical Society, for the observation of total solar eclipses; and the onerous work thrown upon the Society for several years in connection with the National Antarctic Expedition, undertaken jointly with the Royal Geographical Society, which has this year returned home crowned with success as regards the latter; but the Society's labours are not at an end, for the prolonged and responsible task of the discussion and publication of the scientific results of the Expedition is still before them.

In addition to the numerous undertakings, of which some examples have been given, in which the influence and work of the Society have been exercised for national or public objects, there are a number of other ways in which the Society makes its influence continually felt and of which the responsibilities are always with it. The Society is represented by the

President, as an *ex-officio* elector, in the election of eight scientific Professorships at the Oxford University, and one Professorship at Cambridge. The President is also *ex-officio* a trustee of the British Museum, and of the Hunterian Museum, and a Governor of the City and Guilds of London Institute. The Society has a voice, through a representative Fellow chosen by the Council, on the Governing bodies of the Imperial Institute, the Lister Institute of Preventive Medicine, Sir John Soane's Museum, Eton, Rugby, Harrow, Winchester, and four other Public schools, and the Advisory Board for Military Education. The Council of the Society are electors of four members of Lawes' Agricultural Trust, and are nominators of the members of the Meteorological Council. The Society is represented by the President and six of the Visitors on the Board of the Greenwich Observatory. One of the four sets of copies of the Standard Weights and Measures is held in custody by the Society. There is also a Committee for systematic work in Seismology.

To the Royal Society is entrusted the responsible task of administrating the annual Government Grant of £4000 for the purpose of scientific research, and a grant of £1000 in aid of the publication of scientific papers.

In addition to these permanent responsibilities, which are always with the Society, its advice and aid are sought from time to time both by the Government and by Scientific Institutions at home and abroad, in favour of independent objects of a more or less temporary character, of which, as examples, may be taken the recent action of the Society for the purpose of obtaining Government aid for the continuation through Egypt of the African Arc of Meridian, and for the intervention of the Government to assist in securing the fulfilment of the part undertaken by Great Britain in the International Astrographic Catalogue and Chart.

Upon the present Fellows falls the glorious inheritance of unbounded free labour ungrudgingly given during two centuries and a-half for the public service, as well as of the strenuous prosecution at the same time of the primary object of the Society, as set forth in the words of the Charters: "The promotion of Natural Knowledge." The successive generations of Fellows have unsparingly contributed of their time to the introduction and promotion, whenever the opportunity was afforded them, of scientific knowledge and methods into the management of public concerns by Departments of the Government. The financial independence of the Royal Society, neither receiving, nor wishing to accept State aid for its own private purposes, has enabled the Society to give advice and assistance which, both with the Government and with Parliament, have the weight and finality of a wholly disinterested opinion. I may quote here the words of a recent letter from

H.M. Treasury:—"Their Lordships have deemed themselves in the past very fortunate in being able to rely, in dealing with scientific questions, upon the aid of the Royal Society, which commands not only the confidence of the scientific world, but also of Parliament."

In the past the Royal Society has been not infrequently greatly hampered in giving its advice, by the knowledge that the funds absolutely needed for the carrying out of the matters in question in accordance with our present scientific knowledge would not be forthcoming. Though I am now speaking on my own responsibility, I am sure that the Society is with me, if I say that the expenditure by the Government on scientific research and scientific institutions, on which its commercial and industrial prosperity so largely depend, is wholly inadequate in view of the present state of international competition. I throw no blame on the individual members of the present or former Governments; they are necessarily the representatives of public opinion, and cannot go beyond it. The cause is deeper, it lies in the absence in the leaders of public opinion, and indeed throughout the more influential classes of society, of a sufficiently intelligent appreciation of the supreme importance of scientific knowledge and scientific methods in all industrial enterprises, and indeed in all national undertakings. The evidence of this grave state of the public mind is strikingly shown by the very small response that follows any appeal that is made for scientific objects in this country, in contrast with the large donations and liberal endowments from private benefaction for scientific purposes and scientific institutions which are always at once forthcoming in the United States. In my opinion, the scientific deadness of the nation is mainly due to the too exclusively mediæval and classical methods of our higher public schools, and can only be slowly removed by making in future the teaching of Science, not from text-books for passing an examination, but, as far as may be possible, from the study of the phenomena of Nature by direct observation and experiment, an integral and essential part of all education in this country.

I proceed to the award of the Medals.

COPLEY MEDAL.

The Copley Medal is awarded to Sir William Crookes, F.R.S., for his experimental researches in chemistry and physics, extending over more than fifty years. Ever since his discovery of the element thallium in the early days of spectrum analysis, he has been in the front rank as regards the refined application of that weapon of research in chemical investigation. Later, the discrepancies which he found in an attempt to improve weighings, by conducting the operation in high vacua, were tracked out by him to a repulsion arising from radiation, which was ultimately ascribed by theory to the action of the residual gas. This phenomenon, illustrated by the radiometer, opened up a new and fascinating chapter in the dynamical theory of rarefied gases, which the genius of Maxwell, O. Reynolds, and others has left still incomplete. The improvements in vacua embodied in the Crookes tube led him to a detailed and brilliant experimental analysis of the phenomena of the electric discharge across exhausted spaces; in this, backed by the authority of Stokes, he adduced, long ago, powerful cumulative evidence that the now familiar cathode rays, previously described by C. F. Varley, must consist of projected streams of some kind of material substance. His simple but minutely careful experiments on the progress of the ultimate falling off in the viscosity of rarefied gases, from the predicted constant value of Maxwell, at very high exhaustions, gave, in Stokes' hands, an exact account of the trend of this theoretically interesting phenomenon, which had already been approached in the investigations of Kundt and Warburg, using Maxwell's original method of vibrating discs.

These examples, not to mention recent work with radium, convey an idea of the acute observation, experimental skill, and persistent effort, which have enabled Sir William Crookes to enrich physical science in many departments.

RUMFORD MEDAL.

The Rumford Medal is awarded to Prof. Ernest Rutherford, F.R.S., on account of his researches on the properties of radio-active matter, in particular for his capital discovery of the active gaseous emanations emitted by such matter, and his detailed investigation of their transformations. The idea of radiations producing ionization, of the type originally discovered by Röntgen, and the idea of electrified particles, like the cathode rays of vacuum tubes, projected from radio-active bodies, had gradually become familiar through the work of a succession of recent investigators, when Rutherford's announcement of a very active substance, diffusing like a gas with a definite atomic mass, emitted by compounds of thorium, opened up yet another avenue of research with reference to these remarkable bodies. The precise interpretation of the new phenomena, so promptly perceived by Rutherford, was quickly verified for radium and other substances, by various observers, and is now universally accepted. The modes of degradation, and the enormous concomitant radio-activity, of these emanations, have been investigated mainly by Rutherford himself, with results embodied in his treatise on Radio-activity and his recent

Bakerian Lecture on the same subject. It perhaps still remains a task for the future to verify or revise the details of these remarkable transformations of material substances, resulting apparently in the appearance of chemical elements not before present; but, however that may issue, by the detection and description of radio-active emanations and their transformations, Prof. Rutherford has added an unexpected domain of transcendent theoretical interest to physical science.

ROYAL MEDAL.

A Royal Medal is awarded to Prof. W. Burnside, F.R.S., on the ground of the number, originality, and importance of his contributions to Mathematical Science. The section of our "Catalogue of Scientific Papers" for the period 1883–1900, enumerates fifty-three papers by Prof. Burnside, the first dated 1885, and the "International Catalogue of Scientific Literature" thirteen more. His mathematical work has consisted laregly of papers on the Theory of Groups, to which he has made most valuable additions. In 1897 he published a volume "On the Theory of Groups of Finite Order," which is a standard authority on that subject. Two recent papers on the same theory, published in 1903, may be specially mentioned. In one of these he succeeded in establishing by direct methods, distinguished by great conciseness of treatment, the important subsidiary theory of group-characteristics, which had been originally arrived at by very indirect and lengthy processes. In the other he proved quite shortly the important result that all groups of which the order is the product of powers of two primes are soluble.

Besides the treatise and papers relating to group theory, Prof. Burnside has published work on various branches of pure and applied mathematics. His work on automorphic functions dealt with an important and difficult special case which was not included in the theory of these functions as previously worked out. The paper on Green's function for a system of non-intersecting spheres was perhaps the first work by any writer in which the notions of automorphic functions and of the theory of groups were applied to a physical problem. He has also made important contributions to the Theory of Functions, Non-Euclidean Geometry, and the Theory of Waves on Liquids. His work is distinguished by great acuteness and power, as well as by unusual elegance and most admirable brevity.

ROYAL MEDAL.

The other Royal Medal is awarded to Col. David Bruce, F.R.S., who, since 1884, has been engaged in prosecuting to a successful issue researches into the causation of a number of important diseases affecting man and animals. When he went to Malta in 1884 the exact nature of the widely-prevalent "Malta," "Rock," or "Mediterranean" Fever was entirely unknown. After some years' work at the etiology of this disease, he discovered in 1887 the organism causing it, and succeeded in cultivating the *Micrococcus melitensis* outside the body. This discovery has been confirmed by many other workers, and is one of great importance from all points of view, and perhaps more especially as, thanks to it, Malta Fever can now be separated from other diseases, *e.g.*, typhoid, remittent, and malarious fevers, with which it had hitherto been confounded.

During the next few years he was engaged in researches of value on Cholera, and on methods of immunisation against this disease. He also carried out some work on the Leucocytes in the Blood, published in the "Proceedings of the Royal Society," 1894.

In 1894 he was requested by the Governor of Natal to investigate the supposed distinct diseases of "Nagana" and the Tsetse Fly disease. In the short time of two months he made the most important discovery that these two diseases were one and the same, and dependent upon the presence of a protozoan organism in the blood known as a Trypanosoma. Some six months later Bruce was enabled to return to Zululand, and remained there two years, studying the disease and making the discovery that the Tsetse Fly acted as the carrier of the organism which caused it. He was thus the first to show that an insect might carry a protozoan parasite that was pathogenic. This observation was made in 1895.

Bruce not only determined the nature and course of "Nagana," but in addition he studied the disease in a large number of domestic animals, and also observed the malady in a latent form in the wild animals of South Africa. Subsequent observers have found but little to add to Bruce's work on this subject.

In 1900, Bruce was ordered to join a Commission investigating the outbreak of Dysentery in the Army in South Africa, and a great part of the laboratory work performed by this Commission was carried out by him.

In 1903, Col. Bruce went, at the request of the Royal Society, to Uganda, to investigate further the nature of Sleeping Sickness. It was very largely, if not entirely, owing to him that the work of the Royal Society's Commission was brought to a successful issue. At the time when he arrived, a Trypanosoma had been observed by Castellani in a small number of cases of this disease; thanks to Bruce's energy and scientific insight, these observations were rapidly extended, and the most conclusive evidence obtained, that in all cases of the disease the Trypanosoma was present. He showed further that a certain Tsetse Fly, the *Glossina palpalis*, acted as the carrier of the Trypanosoma, and obtained evidence showing that the distribution of the disease and of the fly were strikingly similar.

Bruce has therefore been instrumental in discovering and establishing the exact nature and cause of three wide-spread diseases of man and of animals, and in two of these, Nagana and Malta Fever, he discovered the causal organism. In the third, Sleeping Sickness, he was not the first to see the organism, but he was quick to grasp and work out the discovery, and he made the interesting discovery of the carrier of the pathogenic organism, and thus discovered the mode of infection and of spread of the malady, matters of the highest importance as regards all measures directed to arrest the spreading of the disease. All this research work has been done whilst serving in the Royal Army Medical Corps, and engaged in the routine work of the Service.

DAVY MEDAL.

The Davy Medal is awarded to Prof. W. H. Perkin, jun., F.R.S., for his masterly and fruitful researches in the domain of synthetic organic chemistry, on which he has been continuously engaged during the past twenty-five years.

Dr. Perkin's name is identified with the great advances which have been made during the past quarter of a century in our knowledge of the ring or cyclic compounds of carbon. Thus, in the year 1880, the cyclic carbon compounds known to chemists were chiefly restricted to the unsaturated groupings of six carbon atoms met with in benzene and its derivatives, whilst the number of compounds in which saturated carbon rings had been recognised was very limited, and it was indeed considered very doubtful whether compounds containing carbon rings with more or less than six atoms of carbon were capable of existence.

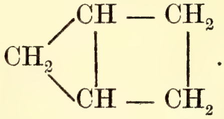
The starting point for Dr. Perkin's researches in this field of enquiry was his investigation of the behaviour of the di-halogen derivatives of various organic radicals with the sodium compounds of malonic, aceto-acetic, and benzoyl-acetic esters, which led to the synthesis of the cyclic polymethylene compounds up to those of hexamethylene, whilst heptamethylene derivatives were obtained by an adaptation of the well-known reduction of ketonic bodies leading to pinacones. The reactions thus introduced by Perkin are now classical, having proved themselves of the highest importance for synthetical purposes and having been instrumental in stimulating the further investigation of the cyclic compounds of carbon.

Dr. Perkin also extended the same methods to the synthetical formation of carbon rings of the aromatic series, obtaining by means of ingeniously designed reactions derivatives of hydrindonaphthene and tetrahydronaphthalene.

But whilst the above achievements depend mainly on happily conceived and brilliantly executed extensions of the malonic and aceto-acetic ester syntheses, Perkin has, by a remarkable development of the Frankland and Duppa reaction for the synthesis of hydroxyacids, been successful in building up the important camphoronic acid in such a manner as to place its constitution beyond doubt (1897).

Dr. Perkin has further devoted much attention to the important subject of the constitution of camphor, towards the elucidation of which he has contributed valuable experimental evidence embodied in a most important and elaborate paper, containing the results of many years' work in conjunction with numerous pupils, entitled "Sulphocamphylic acid and Isolauronolic acid, with remarks on the Constitution of Camphor and some of its derivates" (1898). Bearing on the same subject are later communications on camphoric acid and isocamphoronic acid.

About the year 1900, Perkin, in prosecuting his researches on the constitution of camphor compounds, succeeded in devising synthetical methods for the production of what he has termed "bridged rings," of which a simple example is furnished by the hydrocarbon dicyclopentane



The universal admiration of organic chemists has been called forth by these investigations; they reveal, indeed, a wonderful capacity for devising reactions which coerce carbon atoms to fall into the desired groupings.

Of other publications displaying not only extraordinary experimental skill but close reasoning and the power of interpreting results, mention may be made of Dr. Perkin's memorable researches on the constitution of dehydracetic acid, berberine, brasilin, and hæmatoxylin respectively.

During the present year (1904), Dr. Perkin has made perhaps the most remarkable addition to the long list of his achievements by successfully synthesising terpin, inactive terpineol, and dipentene, substances which had previously engaged the attention of some of the greatest masters of organic chemistry.

In conclusion it may be stated that Professor Perkin is not only the author of the above and numerous other important researches which are outside the scope of this brief summary, but that he has also created a school of research in organic chemistry, which stands in the very highest rank.

DARWIN MEDAL.

The Darwin Medal is awarded to Mr. William Bateson, F.R.S., for his researches on heredity and variation.

Mr. Bateson began his scientific career as a morphologist, and distinguished himself by researches on the structure and development of Balanoglossus, which have had a far-reaching influence on morphological science, and which established to the satisfaction of most anatomists the affinity of the Enteropneusta to the Chordate phylum. Dissatisfied, however, with the methods of morphological research as a means of advancing the study of evolution, he set himself resolutely to the task of finding a new method of attacking the species problem. Recognising the fact that variation was the basis upon which the theory of evolution rested, he turned his attention to the study of that subject, and entered upon a series of researches which culminated in the publication in 1894 of his well-known work, entitled "Materials for the Study of Variation, etc." This book broke new ground. Not only was it the first systematic work which had been published on variation, and, with the exception of Darwin's "Variation of Animals and Plants under Domestication," the only extensive work dealing with it; but it was the first serious attempt to establish the importance of the principle of discontinuity in variation in its fundamental bearing upon the problem of evolution, a principle which he constantly and successfully urged when the weight of authority was against it. In this work he collected and systematised a great number of examples of discontinuous variation, and by his broad and masterly handling of them he paved the way for those remarkable advances in the study of heredity which have taken place in the last few years, and to which he has himself so largely contributed. He was the first in this country to recognise the importance of the work of Mendel, which, published in 1864, and for a long time completely overlooked by naturalists, contained a clue to the labyrinth of facts which had resulted from the labours of his predecessors. He has brought these results prominently forward in England in his important reports to the Evolution Committee of the Royal Society, and in papers before the Royal and other Societies, and also before horticulturists and breeders of animals. He has gathered about him a distinguished body of workers, and has devoted himself with great energy and with all his available resources to following out lines of work similar to those of Mendel. The result has been the supporting of Mendel's conclusions and the bringing to light of a much wider range of facts in general harmony with them. It is not too much to say that Mr. Bateson has developed a school of research to which many biologists are now looking as the source from which the next great advance in our knowledge of organic evolution will come.

SYLVESTER MEDAL.

The Sylvester Medal is awarded to Georg Cantor, Professor in the University of Halle, on account of his researches in Pure Mathematics. His work shows originality of the highest order, and is of the most far-reaching importance. He has not only created a new field of mathematical investigation, but his ideas, in their application to analysis, and in some measure to geometry, furnish a weapon of the utmost power and precision for dealing with the foundations of mathematics, and for formulating the necessary limitations to which many results of mathematics are subject.

In 1870 he succeeded in solving a question which was then attracting much attention—the question of the uniqueness of the representation of a function by Fournier's series. The extension of the result to cases in which the convergence of the series fails, at an infinite number of suitably distributed points, led him to construct a theory of irrational numbers, which has since become classical. From the same starting point he developed, in a series of masterly memoirs, an entirely new branch of mathematics—the Theory of Sets of Points.

Having established the fundamental distinction between those aggregates which can be counted and those which cannot, Cantor showed that the aggregates of all rational numbers and of all algebraic numbers belong to the former class, and that the arithmetic continuum belongs to the latter class, and further, that the continuum of any number of dimensions can be represented point for point by the linear continuum. Proceeding with these researches he introduced and developed his theory of "transfinite" ordinal and cardinal numbers, thus creating an Arithmetic of the Infinite. His later abstract theory of the order-types of aggregates, in connection with which he has given a purely ordinal theory of the arithmetic continuum, has opened up a field of research of the greatest interest and importance.

HUGHES MEDAL.

The Hughes Medal is awarded to Sir Joseph Wilson Swan, F.R.S., for his invention of the incandescent electric lamp, and his other inventions and improvements in the practical applications of electricity. Not as directly included in the award, should be mentioned his inventions in dry-plate photography, which have so much increased our powers of experimental investigation.

Researches on some of the Physiological Processes of Green Leaves, with special Reference to the Interchange of Energy between the Leaf and its Surroundings.

By HORACE T. BROWN, LL.D., F.R.S., and F. ESCOMBE.

Received January 9,—Read March 23, 1905.

(This paper, and the three following ones, constituted the BAKERIAN LECTURE for 1905.)

PART I.—DESCRIPTION OF APPARATUS AND METHODS.

Introductory.

In the following series of five papers we have endeavoured to bring together in a connected form the results of various researches carried out in the Jodrell Laboratory at the Royal Gardens, Kew, between the years 1898 and 1901. The present account must be regarded as being supplemented by two previous publications; one on the "Static Diffusion of Gases and Liquids in relation to the Assimilation of Carbon and Translocation in Plants";[*] and the other on "The Influence of varying Amounts of Carbon Dioxide in the Air on the Photosynthetic Process of Leaves."[†]

The main object of the research was, in the first place, to obtain a direct measure of the rate of photosynthesis in a leaf, when it is surrounded by an atmosphere containing an amount of carbon dioxide not far removed from the normal amount of 0·03 per cent.; and secondly to obtain more definite

[*] 'Phil. Trans.,' B, vol. 193 (1900), p. 223.

[†] 'Roy. Soc. Proc.,' vol. 70 (1902), p. 397.

information on the "energetics" of the leaf, especially as regards its power of absorbing and transforming the solar radiation incident upon it.

Description of Apparatus and Methods.

In any investigation of the phenomena of assimilation which is based on a direct determination of the rate of intake of the carbon dioxide of ordinary atmospheric air it is evident that, in order to ensure success, the experiments must be carried out on a relatively large scale, both as regards the area of leaf-surface exposed, and the volume of air passed through the apparatus. Another essential condition is that we must have an accurate but not too laborious method for determining the amount of carbon dioxide in the air both before and after contact with the assimilating leaf.

After a considerable amount of preliminary work we adopted the following arrangement of apparatus, which has been used throughout this research, and has proved well adapted to the purpose for which it was devised.

The leaf under experiment is enclosed in a flat rectangular case (A, fig. 1) consisting of a light wooden frame glazed on two sides. The wooden strips before being dove-tailed together to form the frame are carefully dried, and soaked for some time in a bath of hot melted paraffin, in order to render the wood impermeable to air.

The glass plate on one side of the case is permanently fixed, but that of the opposite side is moveable, and drops into a rebate in the frame, into which it may be fixed air-tight with soft wax-mixture after introducing the leaf.*

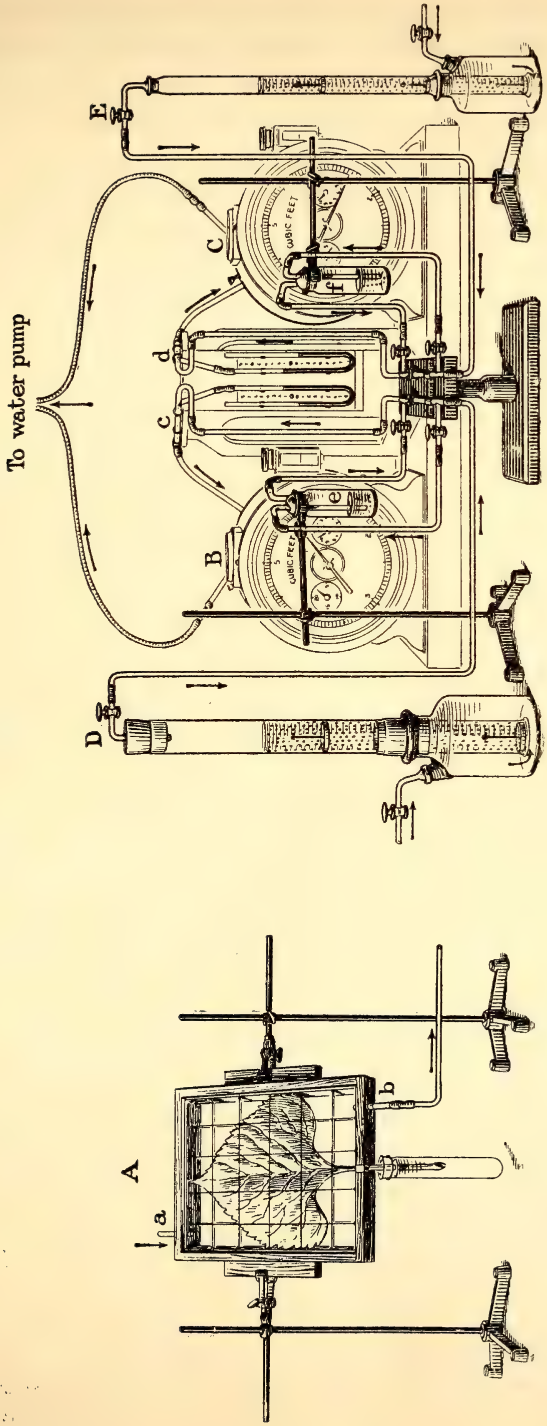
In order to maintain the leaf-lamina parallel to the glass sides of the case and to ensure sufficient space for the circulation of the air on both sides, the leaf rests on threads which are laced through small metal eyes screwed into the frame at intervals of about half an inch, and a similar lacing of threads above the leaf serves to fix it in the right position.

If a detached leaf is employed, the petiole passes through an aperture in the side of the case, and dips into a small tube outside, which is filled with water. If, on the other hand, it is desired to experiment on a leaf which is still attached to its plant, there is a slot cut in the wooden frame to about half its depth, into which fits a moveable tongue of wood so arranged that when the leaf is in position the tongue nearly fills up the slot. The spaces around the leaf-stalk and the tongue are made air-tight with the luting mixture already alluded to.

---

* The wax luting employed was that recommended by F. F. Blackman, 'Phil. Trans.,' B, vol. 186 (1895), p. 522.

Fig. 1.


To water pump
E
C
d
c
B
D
f
e
CUBIC FEET
5
A
a
b

These cases were made in several sizes according to requirements, the largest being capable of taking a fair-sized leaf of the sunflower. An inlet tube *a* for the air is fixed through one side of the frame, and a corresponding outlet *b* on the opposite side, the tubes being respectively above and below the plane of the leaf.

The necessary current of air is drawn through the case by a water-pump, the volume being determined by carefully-standardized meters B and C reading to 20 c.c., and since the volumes of air employed vary from about 200 to 900 litres or more, errors of measurement are practically non-existent.

The air on its way from the leaf-cases to the meters has to pass through the absorption-apparatus D and E presently to be described, and as this introduces a resistance, the air enters the meters at less than atmospheric pressure. This difference of pressure is measured by the mercury manometers *c* and *d* in connection with the inlet to the meters. During an experiment frequent observations are made of these manometers, and also of the barometric pressure and the temperature of the air around the meters, the necessary data for reducing the volumes of air to normal temperature and pressure being thus obtained.

In the glass connection between the absorption-apparatus and the meter there is a small by-pass so arranged with glass taps that a portion of the air-stream can be deflected through small vessels *e* and *f* containing baryta-water.

These act as "guard-tubes," and if the baryta-water remains perfectly clear throughout the experiment, it is an indication that the absorption of carbon dioxide from the air-stream has been complete.*

The apparatus used for the absorption of the carbon dioxide from the air-stream is similar to the one used by Reiset in his estimations of the carbon dioxide of the atmosphere.†

It is shown in its original form at D in the figure, and consists essentially of a long wide glass tube fixed vertically by means of an india-rubber cap into a wide-mouthed glass vessel furnished with a second opening through which the air enters the apparatus. The height of the tube is always 50 cm., but its width may vary according to the amount of air which has to be passed in a given time.

The lower end of the vertical tube, which is adjusted within a few millimetres of the bottom of the glass vessel, is closed with a silver plate pierced with fine holes about 0·5 mm. in diameter. There should be not less than ten of these holes per square centimetre.

---

* The absorptive power of the Reiset's apparatus, when due precautions are taken, is so very complete that this portion of the apparatus is almost superfluous.

† 'Compt. Rend.,' vol. 88, p. 1007, and vol. 90, p. 1144; see also Hempel's "Gas Analysis," p. 83.

Two other similar perforated plates are fixed in the vertical tube at heights of about 12 and 25 cm. respectively, and the upper part of the tube is closed with a perforated india-rubber plug, through which passes a tube connecting with the meter. The side-tubulure of the glass vessel is connected either with the leaf-case A or with the outer air as may be required, the liquid for absorbing the carbon dioxide being placed in the glass vessel. When the water-pump is in action the absorbent liquid rises in the vertical tube to a height of about 30 cm., after which air is drawn through the lowest perforated plate and rises through the column of liquid in a rapid stream of small bubbles which are broken up and re-formed at each of the two succeeding plates, thus producing a very effective "scrubbing" action.

The absorption-tubes we have employed are of two sizes, one of them having a diameter of 40 mm. (shown at D of fig. 1) and applicable to rates of flow of from 200 to 400 litres per hour, and another smaller apparatus (shown at E of fig. 1) with a tube diameter of 20 mm. and suitable for volumes of air of from 100 to 150 litres per hour.

This last mentioned apparatus we have had made with glass connections throughout. In this form it is well adapted for the determination of carbon dioxide in the air, and it has the advantage that the whole of the liquid can be used for the final titration instead of an aliquot part as in the case of the larger apparatus.

The apparatus as figured is arranged for the determination of the carbon dioxide of the air, for which the smaller Reiset's apparatus E is used, whilst the larger Reiset D is connected with the current of air coming from the experimental leaf-case. The water-pump is not shown in the illustration.

Instead of baryta, as originally employed by Reiset, we use a 4-per-cent.-solution of pure caustic soda (made from sodium) as the absorbent.

After displacing the air in the absorption-apparatus with air freed from carbon dioxide* a definite volume of the soda-solution is run into the "Reiset" through the side opening of the large vessel, every precaution being taken to avoid absorption of atmospheric carbon dioxide during the process. The larger apparatus requires 400 c.c. and the smaller 100 c.c. of the solution.

This apparatus acts as a most efficient absorber of the carbon dioxide of ordinary air, and of air artificially enriched with that gas up to ten or twenty times the normal amount. We have made an exhaustive series of test-experiments on this point and have found by introducing a second Reiset apparatus "in series" that there is no falling-off of complete absorption in the first apparatus until from one-fifth to one-fourth of the alkali has been carbonated, and that even when this point is reached the falling-off of absorption is due to a virtual shortening of the column of alkaline solution by the excessive carbonation of its lowest section; for when the contents of the three "stages" of the tube are thoroughly mixed the column once more becomes completely absorbent. In actual practice only a very small fraction of the total amount of caustic alkali is carbonated, and providing the column of liquid is not less than 50 cm. in length the speed of the air-current is practically only limited by the danger attending the ejection of liquid from the tube.

---

* The Winkler apparatus is used for this purpose, the air being driven through it with an ordinary india-rubber ball-pump.

At the completion of an experiment, if the whole amount of alkali has to be submitted to the method of "double titration," which is always the case with the smaller apparatus, the vertical tube is removed from the glass vessel, rinsed out with a little water free from CO_2, and over the aperture is slipped an india-rubber cap furnished with a tubulure through which the end of the "acid-burette" can be afterwards passed for titration. In using the larger apparatus it was found more convenient to titrate only an aliquot part of the solution; and here we are met with a practical difficulty which must be referred to somewhat in detail. During the passage through the Reiset's "tower" of large volumes of air only partially saturated with moisture, a certain amount of evaporation necessarily takes place, so that the final volume of the absorbing liquid is somewhat less than the initial volume. In addition to this it is necessary to use a little water to wash the upper part of the "tower," owing to the breaking bubbles of the liquid spurting a little of the solution of soda on to the sides of the tube. The final volume of liquid can be determined from the difference in weight of the apparatus at the beginning and end of the experiment, and the known volume of the original alkaline solution taken. If we represent the original volume of the alkaline solution in cubic centimetres by V, and the difference between the initial and final weight in grammes of the charged apparatus by D, then $V+D$ represents the final volume of the solution in cubic centimetres if no contraction of volume has taken place on adding the wash-water. Theoretically such a contraction undoubtedly takes place, but it cannot appreciably affect the result since it is the special merit of the method of titration employed that an error in the determination of the volumes at any stage of the experiment can only affect the final carbon dioxide estimation in the proportion which this error of measurement bears to the true volume. In the large Reiset's apparatus we always employed 400 c.c. of the solution of soda, so that if the accumulated errors of measurement amounted to as much as 0·5 c.c., a very unlikely value, the final estimation of the carbon dioxide could only be affected to the extent of $\frac{0{\cdot}5 \times 100}{400} = 0{\cdot}12$ per cent. of the true amount. When the amount of liquid abstracted from the large apparatus for the final titration is 100 c.c., as is usually the case, the relation which this bears to the original solution is expressed by $V \Big/ \frac{100 \times V}{V'}$, where V represents the initial, and V′ the final volume.

The mode of transferring this 100 c.c. from the Reiset's apparatus to the vessel for titration requires a word of explanation. After rapidly washing down the upper end of the wide tube the plug or stopper is once more inserted, the exit-tube being guarded with a small soda-lime tube. Air, freed from carbon dioxide by passage through a Winkler's absorption-apparatus, is then pumped into the apparatus through one of the tubulures of the lower vessel by means of an india-rubber ball-pump, and the alkaline solution is driven into the wide tube several times in succession until the solution is thoroughly mixed.

After weighing the apparatus a small tube reaching to the bottom of the vessel is inserted into the second tubulure, the upper part of this tube being then connected with a large burette which is filled from below by forcing air down the wide vertical tube. The titration vessel, into which the 100 c.c. of solution is now run from the burette, is a glass cylinder of suitable dimensions furnished with a rubber cap having two apertures through which the ends of the titration-burettes can be passed. In all these operations care is taken that no air can come into contact with the alkaline solution unless it has been previously freed from carbon dioxide by passing through the Winkler's apparatus, and all the open ends of the tubes and burettes are guarded with soda-lime tubes.

The Titration Method.

Although this has already been described in connection with our work on the static diffusion of gases,* it is desirable to give a further account of the method in view of its importance in investigations such as we are considering.

There are grave disadvantages connected with any method of carbon dioxide absorption by means of barium hydroxide when the volume of the solution is large and the estimation has to be deduced from the initial and final titration of the liquid. In such a case it is manifest that we have to depend on the determination of *small differences* between two large values, and that the full acid-equivalent of all errors of measurement of the respective solution-volumes will accumulate in the final result, as well as all other errors incident to methods of titration generally. We therefore discarded the barium hydroxide method at an early stage of the enquiry, and turned our attention to one which is free from the cumulative errors to which we have referred. Such a one is found in a method which was originally proposed by P. Hart in 1887,* for the estimation of the relative amounts of caustic soda and sodium carbonate in soda-ash. As far as we know, this has never been regarded from any other point of view than that of a convenient commercial method, whereas when certain precautions are taken it is capable of a high degree of precision, and really affords one of the most accurate means of determining small quantities of carbon dioxide short of actual measurement of the gas.

---

* 'Phil. Trans.,' B, vol. 193 (1900), p. 289.

It is based on *double titration* with two indicators, one of them phenol-phthalëin, sensitive to free carbon dioxide, the other, methyl-orange, only reacting with the excess of mineral acid used in the titration.

If we have a solution of caustic soda which has become partially carbonated, the first stage of the process is the addition of the phenol-phthalëin-indicator, followed by a mineral acid (hydrochloric) until the pink colour just disappears. This marks the point of the complete conversion of the neutral into the acid carbonate, or more strictly speaking, the point at which the liberation of the first trace of carbon dioxide takes place. Up to this stage it is unnecessary to take any account of the actual amount of acid used, since this is the starting point for the true titration-process.

Methyl-orange is now added, and this is followed by 1/10 normal hydrochloric acid until the whole of the acid carbonate is decomposed, the volume of the 1/10-normal acid requisite to produce this effect, being of course a measure of the carbon dioxide displaced.

It will be noticed that this method is quite independent of the relations of the total acid-equivalent of the alkali before and after absorption of carbon dioxide, the determination of the carbon dioxide absorbed merely depending on the amount of dilute acid required in each case between the first reaction with phenol-phthalëin and the second with methyl-orange. There is also the great advantage that errors in the estimation of the volumes of the solutions will not be accumulated in the result, but will only affect those results in the proportion the errors of measurement bear to the true volume.

---

* 'Journ. Soc. Chem. Ind.,' 1887, p. 347.

Certain precautions are necessary if the highest possible degree of accuracy is required.

In order to avoid an undue increase in the volume of the liquid undergoing titration, which would of course diminish the sensitiveness of the reaction, it is advisable to use in the early stages of the first titration an acid of a strength varying, according to circumstances, between normal and 6-normal, until the pink colour of the phenol-phthalëin becomes somewhat faint: this is followed by 1/10-normal acid to the complete disappearance of the colour. Whilst the stronger acid is being run in it is advisable to keep the solution in constant rotation, otherwise acid carbonate is locally decomposed and carbon dioxide evolved, a misfortune which cannot be rectified.

Caustic soda made from metallic sodium should alone be used, since a trace of alumina or iron interferes with the sharpness of the second titration. A trace of alkaline silicate also interferes with the delicacy of the phenol-phthalëin reaction, a fact which Letts and Blake have also observed,* but this is not so important a drawback when the above double-titration method is used as it is in methods which are dependent on the exact titration of the whole of the alkali at start and finish.

The actual titrations in our experiments were carried out in cylindrical vessels covered with an india-rubber cap furnished with two tubulures through which the delivery-tubes of the burettes were passed. The use of this cap is unnecessary for the second titration. The burettes used for the soda-solution are filled from stock-bottles through side-tubes, and are furnished at the top with guard-tubes of soda-lime.

In determining the final point in the first titration the meniscus of the liquid is carefully observed, since residual colour can be seen there when it is quite inappreciable in the body of the liquid.

For the second titration a colour-control was always used, containing the same amount of methyl-orange as the liquid titrated, and brought to a constant arbitrary tint of acidity.

When all these precautions are taken the method is sensitive to extremely small differences of carbon dioxide content in the soda-solution employed. In the paper already cited † we have given a series of experiments made with a view to test its limits of delicacy. There is no difficulty in determining in 100 c.c. of an alkaline solution differences in the amounts of carbonate corresponding to from 0·1 to 0·15 of a cubic centimetre of carbon dioxide. This corresponds approximately to the carbon dioxide in from 350 to 500 c.c. of air, and since not less than 400 litres of air is passed through the absorption-apparatus for every 100 c.c. of soda-solution, the percentage-error of titration on the amount of carbon dioxide estimated is not more than 0·08 to 0·12 *per cent.* of the true amount.

---

* 'Dublin Soc. Proc.,' vol. 9 (1900), p. 152.

† 'Phil. Trans.,' B (1900), p. 291.

Determination of the Leaf-Area.

The most convenient plan for determining leaf-areas with exactness is to place the leaves at the close of an experiment in contact with sensitized paper in an ordinary photographic printing-frame and expose them to light for a short time. The outline of the leaf-print is then followed with an Amsler's planimeter set to read off square centimetres. This is a far more accurate and rapid method than that of cutting out a facsimile of the leaf from paper of a known weight per unit area.

Apparatus for Increasing the Amount of Carbon Dioxide in Air.

If it is desired to determine the assimilative power of a leaf in an atmosphere artificially enriched with carbon dioxide, we employ a form of apparatus which is a modification of the generator described by Blackman.[*] The air-stream before entering the leaf-case is passed through a small tower containing fragments of marble over which is slowly dropped very dilute acid at such a rate as to give approximately the desired amount of carbon dioxide to the air current. Knowing the strength of the acid employed and the volume of air passed, it is easy to adjust the rate of flow of the acid approximately to the desired point.

The stream of air as it leaves this apparatus is divided, one part of the split current passing through the experimental leaf-case, whilst the other passes direct to a Reiset's absorption apparatus and separate meter for the exact estimation of the carbon dioxide it contains.

Rotating Sectors.

In those cases where it is desired to vary at will, and in a known ratio, the amount of radiant energy falling on a leaf, it is necessary to use some kind of screen which will arrest equal proportions of all the undulations of varying wave-length; in other words the screen must exercise no "selective absorption" on the radiations.

---

[*] 'Phil. Trans.,' vol. 186 (1895), p. 495.

The most satisfactory way of meeting this difficulty is to employ the method of *rotating sectors* which has been largely used by Abney in his investigations on colour-measurement. For this purpose the Cambridge Scientific Instrument Company constructed for us an apparatus consisting of a series of moveable metal sectors, which could be adjusted on a revolving axis in such a manner as to cut off from an object placed under the sectors any desired amount of the total solar radiation falling on it.

The motive power was, in our case, supplied by a small water turbine, but the use of a small electric motor would much simplify the construction. The broken disc, formed by the sectors, could be inclined at any desired angle, and when rotated in front of a leaf, placed parallel to it, cut off a perfectly definite proportion of the solar radiation, and the effect could be compared with that produced by the full radiation falling on another leaf alongside the first.

Measurement of the Intensity of Radiation.

The intensity of radiation falling on the leaf-surface was determined by means of a Callendar's radiometer, consisting of a pair of differential platinum thermometers, the one black and the other bright, wound on flat plates of mica and placed side by side in a flat rectangular glazed case, which was mounted on an adjustable stand so that the radiometer could be placed alongside the leaf and in exactly the same plane.

The platinum coils occupy an area of about 75 sq. cm., and the difference in temperature between them, which is proportional to the intensity of the vertical component of the radiation, is determined by connecting them with a Callendar's recorder, consisting essentially of a "Wheatstone's bridge or potentiometer in which the movement of the slider along the bridge-wire is automatically effected by relays, worked by the current passing through the galvanometer between the bridge-arms."*

A simple form of planimeter attached to the instrument integrates the curve drawn by the pen on the revolving drum, and from the known constants of the instrument the planimeter-readings can be readily converted into water-gramme-units (calories) per square centimetre per minute, due regard being paid to any extra resistances which it may be necessary to insert during the course of the experiment for the purpose of keeping the pen within the range of the drum.

We are much indebted to Professor Callendar for his assistance and advice in carrying out the preliminary work connected with this instrument, and especially for the labour he bestowed on the calibration of the radiometer in absolute thermal units.

---

* Professor Callendar described his recorder in detail in 'Engineering' for May 26, 1899. For a general description of the Callendar's radiometer, see 'British Association Report' for 1898, p. 796.

The radiometer and recorder were constructed by the Cambridge Scientific Instrument Company, and their cost was defrayed by the Government Grant Committee of the Royal Society who kindly put the instruments at our disposal for this research.

When a bridge-wire No. $\frac{1}{2}$ is used in the recorder of this particular instrument, 100 scale-divisions = 1 ohm, and with the radiometer in sunshine in the horizontal position, 1 scale-division (= 4 mm.) is equivalent to a radiation of 0·0070 calorie per sq. cm. per minute. With the radiometer in bright sunshine and inclined at an angle of 45° to the horizon, 1 scale-division = 0·0074 calorie per sq. cm. per minute, the difference in this case being due to the influence of air-convection on the coils.

PART II.—EXPERIMENTS ON ASSIMILATION AND RESPIRATION.

CONTENTS.

PAGE
Section (1).—The Rate of Assimilation of Leaves as deduced from the Direct Estimation of the Carbon Dioxide absorbed in the Photosynthetic Process 40
Section (2).—A Comparison of the Direct Method of Determining the Rate of Assimilation in Ordinary Air with the Weighing Method of Sachs.............................. 49
Section (3).—The Relation of the Distribution of Stomata to the Rate of Gaseous Exchange in the Leaf 61
Section (4).—(*a*) Experiments on Leaves exposed to Artificial Illumination in a Current of Ordinary Air 66
(*b*) Experiments on the Respiration of Leaves.. 68

Section (1).—*The Rate of Assimilation of Leaves as deduced from the Direct Estimation of the Carbon Dioxide absorbed in the Photosynthetic Process.*

In a previous paper dealing with the influence of varying amounts of carbon dioxide on the photosynthetic process of leaves,[*] it was shown that, providing there is an excess of radiant energy of the right quality incident on the leaf, *the rate at which photosynthesis takes place is approximately proportional to the mean partial pressure of the carbon dioxide* in the air supplied to the leaf during the experiment.

Another way of expressing this fact is as follows; If we denote the partial pressure of the carbon dioxide of ordinary air by p, that of artificially-enriched air by p', the rate of assimilation in ordinary air by a, and that in enriched air by a'; then, within certain limits, $p/a = p'/a'$.

---

[*] 'Roy. Soc. Proc.,' vol. 70 (1902), p. 397.

Our experiments have shown that this relation of partial pressure to assimilation certainly holds good up to 15 parts of carbon dioxide per 10,000 of air, that is to say, up to a concentration of five times the normal amount; but it is highly probable that this by no means expresses the upward limit of the application of this rule, which is one of considerable practical importance when we desire to determine the true rate of assimilation in ordinary air, as will be seen from the following considerations.

The actual amount of carbon dioxide which has been abstracted from a measured volume of air passed over the leaf is expressed by the difference in the amount of carbon dioxide in the air on entering and emerging from the leaf-case, and since the mean carbon dioxide-content of the air in contact with the leaf must necessarily be less than that of the outer air, a correction must be made if we desire to ascertain the true rate at which the leaf would assimilate under free-air conditions, all other conditions being constant. The above rule enables us to make such a correction, the magnitude of which will be readily seen from the following concrete example taken from an actual experiment.

A leaf of *Catalpa bignonioides* having an area of 157·45 sq. cm. was enclosed in its glazed case and submitted to intermittent sunshine for six hours, during which time 322·57 litres of air were passed over it. From an analysis of the ingoing and outgoing air it was found that the leaf had abstracted 32·58 c.c. of carbon dioxide (at 0° and 760 mm.), which is equivalent to an assimilation of 3·448 c.c. per square decimetre per hour. Since, however, the outer air as it entered the leaf-case contained 2·80 volumes of carbon dioxide per 10,000 volumes of air, whilst the emerging air contained only 1·79 volumes, the mean carbon dioxide-content of the air in contact with the leaf was only $\frac{2{\cdot}80 + 1{\cdot}79}{2} = 2{\cdot}295$ per 10,000. Hence, from the above rule of partial pressures, the amount of carbon dioxide which the leaf would have assimilated in the outer air, all other conditions being the same, is $\frac{3{\cdot}448 \times 2{\cdot}8}{2{\cdot}295} = 4{\cdot}206$ c.c. of carbon dioxide per square decimetre per hour.

The correction in this case, therefore, which is a somewhat extreme one, is +22 per cent.

Although such a method as we are considering ought to give, with a fair approximation to accuracy, a measure of the *effective assimilation* going on in the leaf, the results are subject to a further correction if we desire to know the exact amount of photosynthetic work accomplished, since there is active respiration going on in the leaf-cells, and the carbon dioxide due to this process is not evolved, but undergoes re-elaboration under the influence of the light. The intensity of the respiratory function increases, within certain limits, with the temperature. Under the conditions of the particular experiment just cited the re-synthesised carbon dioxide of respiration amounted to about 0·5 c.c. per square decimetre per hour, so that the total photosynthetic work accomplished is represented by $4·206 + 0·5 = 4·706$ c.c. of carbon dioxide per square decimetre per hour, being a further increase of about 11 per cent.

In experiments where it is only required to know the *effective assimilation*, that is to say, that portion of the process which can alone contribute to an actual increase in weight of formative carbohydrate in the plant, this correction need not be applied.

We may now consider the results of a series of experiments carried out on the lines indicated, and for the purpose of determining the rate of the effective assimilation of leaves in air containing the normal amount of carbon dioxide.

The volumes of air and carbon dioxide are reduced to normal temperature and pressure (N.T.P.) and the carbon dioxide which has been fixed by the leaf is corrected to the partial pressure of that gas existing in the outer air at the time of the experiment.

The comparatively large scale on which these experiments have been carried out is perhaps best realised by reference to Columns (3) and (6) in the following table, which give respectively the area of the leaf employed and the number of cubic centimetres of carbon dioxide absorbed and utilised by the leaf during the full time that the experiment lasted, amounting in some cases to upwards of 140 c.c. of the gas.

Column (7) gives the volume of carbon dioxide (measured at normal temperature and pressure) which would have been assimilated by 1 square decimetre of leaf per hour, under a partial pressure equivalent to that of the CO_2 in the outer air at the time, and from this value we can readily deduce the actual weight of carbohydrate synthesised for unit-area and unit-time, provided we know the mean empirical formula of the carbohydrate.

If the carbohydrate is a hexose (*e.g.*, *glucose* or *fructose*) or one with the general formula $C_nH_{2n}O_n$, then the absorption by the leaf of one part by weight of carbon dioxide corresponds to the synthesis of 0·681 parts of the carbohydrate. If, on the other hand, we assume that it is *cane-sugar* which is synthesised, or any sugar of the $C_{12}H_{22}O_{11}$ class, then the disappearance of one part by weight of carbon dioxide corresponds to the synthesis of 0·647 parts of the body in question.

Finally, if we assume that *starch* or *inulin* is the synthesised product, having the formula $C_6H_{10}O_5$, then one part by weight of carbon dioxide can give rise to 0·613 parts of the carbohydrate.

In considering which factor we ought to take for calculating the increase in dry weight of the leaf from the amount of carbon dioxide fixed, we must bear in mind that the question is quite independent of the nature of the primary product of assimilation, and turns on the relative proportion of the above three classes of carbohydrates at the close of the experiment.

In two separate determinations of the starch and sugars of leaves of *Tropæolum majus* in full assimilation, Brown and Morris found* them apportioned as follows, the determinations being expressed as percentages on the dry weight of the leaves.

	I.	II.
Starch	3·693	5·425
Cane-sugar	9·980	7·330
Dextrose	0·000	0·000
Lævulose...............	1·410	2·110
Maltose	2·250	2·710
Total per cent. ...	17·333	17·575

By multiplying each one of these values by the factor appropriate to the particular class of carbohydrate as stated above, we obtain average values as follows:—

Factor deduced from I	0·642
„ „ II	0·640

These are not far removed from the arithmetical mean of the three values for the three different classes of carbohydrates, which is 0·646.

The "carbohydrate-factor" we have used for converting carbon dioxide assimilated into its equivalent by carbohydrate is 0·640.

Since 1 c.c. of carbon dioxide at normal temperature and pressure weighs 0·00196, the number of c.c. given in Column (7), Table I, multiplied by (0·00196 × 0·640) should give *the corresponding weight of carbohydrate produced in one square decimetre of leaf per hour.* These values are shown in the last column of Table I.

Before drawing any conclusions from the above results as to the rate of assimilation in ordinary air of the usual carbon dioxide-content, we will give the details of a further set of experiments in which the air supplied to the leaf-cases had been previously enriched with a further determinate amount of that gas.

---

* 'Journ. Chem. Soc.,' 1893, Trans., p, 671.

Table I.—Assimilation in Ordinary Air.

(1) Description of leaf and general conditions.	(2) Temperature C.	(3) Area of leaf in square centimetres.	(4) CO_2 in air, parts per 10,000.		(5) Duration of experiment in hours.	(6) CO_2 absorbed during experiment in cubic centimetres, N.T.P.	(7) CO_2 assimilated per square decimetre of leaf per hour in cubic centimetres.	(8) Carbohydrate assimilated per square decimetre per hour in grammes.
			Mean CO_2 of air in leaf case.	CO_2 in outer air.				
(1) *Helianthus annuus.* 25/8/98. Attached to plant. Strong diffused light of northerly sky ...	21° ·1	743 ·1	2 ·22	2 ·80	2 ·0	36 ·89	**3 ·126**	0 ·00392
(2) *Helianthus annuus.* 29/8/98. A detached leaf. Diffused light of northern sky	19° ·0	743 ·1	1 ·92	2 ·80	6 ·41	143 ·68	**4 ·399**	0 ·00551
(3) *Helianthus annuus.* 25/8/99. A detached leaf. Strong diffused light, under thin canvas screen..................................	21° ·1	312 ·1	2 ·33	2 ·80	4 ·0	29 ·97	**2 ·885**	0 ·00361
(4) *Helianthus annuus.* 25/8/99. Detached leaf. Strong diffused light under canvas screen	26° ·8	336 ·4	2 ·25	2 ·80	4 ·0	41 ·67	**3 ·853**	0 ·00483
(5) *Helianthus annuus.* 29/8/99. Detached leaf. Day cloudy, but strong diffused light............	19° ·4	275 ·9	2 ·29	2 ·80	3 ·25	29 ·37	**4 ·005**	0 ·00502

(6) *Helianthus annuus.* 7/8/00. Leaf still attached to plant. Intermittent sunshine. No screen	22° ·1	760 ·1	2 ·02	2 ·71	4 ·9	79 ·50	**2 ·863**	0 ·00359
(7) *Helianthus annuus.* 11/8/00. Leaf still attached to plant. Bright sunshine. No screen	47° ·1	881 ·2	2 ·72	2 ·79	3 ·7	6 ·98	**0 ·219**	0 ·00027

N.B.—The temperature of the leaf-case was very high, but the leaf still remained turgid. It will, however, be noted that effective assimilation was almost in abeyance, owing to the high temperature.

(8) *Helianthus annuus.* 17/8/00. Leaf still attached to plant. Sunshine. No screen used	41° ·8	979 ·6	2 ·93	2 ·82	4 ·2	nil	nil	nil

N.B.—Owing to the high temperature effective assimilation was entirely in abeyance, respiration being predominant, as shown by emergent air containing more CO_2 than entering air.

(9) *Tropæolum majus.* 4/9/00. A detached leaf. Diffused sunlight through canvas screen	21° ·7	130 ·1	2 ·705	2 ·86	3 ·3	6 ·43	**1 ·583**	0 ·00198
(10) *Tropæolum majus.* 7/9/00. Detached leaf. Sunshine. Case under canvas	25° ·9	193 ·5	2 ·345	2 ·75	5 ·0	20 ·11	**2 ·437**	0 ·00305
(11) *Tropæolum majus.* 11/9/00. Conditions same as last	24° ·9	204 ·0	2 ·57	2 ·80	4 ·85	12 ·03	**1 ·324**	0 ·00166
(12) *Tropæolum majus.* Conditions same as last	24° ·2	169 ·7	2 ·725	3 ·00	4 ·9	14 ·57	**1 ·929**	0 ·00241

Table I.—Assimilation in Ordinary Air—*continued.*

(1) Description of leaf and general conditions.	(2) Temperature C.	(3) Area of leaf in square centimetres.	(4) CO_2 in air, parts per 10,000.		(5) Duration of experiment in hours.	(6) CO_2 absorbed during experiment in cubic centimetres, N.T.P.	(7) CO_2 assimilated per square decimetre of leaf per hour in cubic centimetres.	(8) Carbohydrate assimilated per square decimetre per hour in grammes.
			Mean CO_2 of air in leaf case.	CO_2 in outer air.				
(13) *Tropæolum majus.* Detached leaf. Strong diffused light through canvas screen	20°·6	134·2	2·665	2·91	5·3	12·56	**1·928**	0·00241
(14) *Tropæolum majus.* Two detached leaves, **A** and **B**, exposed simultaneously. Sunshine. Under canvas screen—								
Leaf A	22°·0	130·3	2·70	2·85	5·0	8·66	**1·403**	0·00176
Leaf B	21°·8	137·1	2·66	2·85	5·0	11·07	**1·730**	0·00217
(15) *Catalpa purpurea.* 26/7/98. A detached leaf, exposed to intermittent sunshine...........	22°·7	304·21	2·415	2·92	4·5	26·22	**2·315**	0·00290
(16) *Catalpa bignonioides.* 31/8/99. Detached leaf. Intermittent sunshine	20°·0	417·6	2·05	2·80	4·0	45·65	**3·732**	0·00468
(17) *Petasites albus.* 6/6/00. Detached leaf under canvas screen in sunlight	19°·8	285·9	2·52	2·82	3·2	23·46	**2·869**	0·00359

(18) *Petasites albus.* 26/6/00. Detached leaf. Intermittent sunshine	17° ·0	295 ·0	2 ·56	2 ·84	5 ·3	29 ·38	**2 ·084**	0 ·00261
(19) *Petasites albus.* 5/7/00. Detached leaf. Intermittent sunshine. Screen used part of time	25° ·2	256 ·1	2 ·59	2 ·86	5 ·3	34 ·035	**2 ·769**	0 ·00347
(20) *Polygonum Weyrichii.* 19/6/00. Detached leaf	21° ·0	305 ·2	2 ·24	2 ·82	5 ·0	57 ·36	**4 ·732**	0 ·00593
(21) *Polygonum Weyrichii.* 22/6/00. Detached leaf. Canvas screen in sunshine	19° ·3	378 ·6	2 ·38	2 ·84	4 ·2	48 ·66	**3 ·651**	0 ·00458
(22) *Polygonum Weyrichii.* 3/7/00. Detached leaf under canvas screen in intermittent sunlight	28° ·3	415 ·1	2 ·40	2 ·93	5 ·1	48 ·08	**2 ·772**	0 ·00347
(23) *Polygonum Weyrichii.* 11/7/00. Detached leaf under canvas screen in bright sunshine. Very hot day	37° ·9	384 ·1	2 ·61	2 ·84	4 ·95	28 ·12	**1 ·609**	0 ·00201

N.B.—Effective assimilation has been reduced in this experiment by high temperature.

Table II.—Assimilation in Air containing more than the Normal Amount of Carbon Dioxide.

Description of leaf and general conditions.	(1) Temperature, C.	(2) Area of leaf in square centimetres.	(3) Mean CO_2 content of air in contact with leaf. Parts per 10,000.	(4) Duration of experiment in hours.	(5) CO_2 absorbed during experiment in cubic centimetres at N.T.P.	(6) CO_2 assimilated per square decimetre per hour in cubic centimetres.	(7) Carbohydrate assimilated per square decimetre per hour in grammes.
(1) *Helianthus annuus.* 18/8/98. Leaf still attached to plant. Continuous sunshine	27° ·0	617 ·5	16 ·41	4 ·41	622 ·9	**22 ·87**	0 ·02869
(2) *Helianthus annuus.* 23/8/98. Facing bright northerly sky. Leaf attached to plant	23° ·7	863 ·7	14 ·82	2 ·75	428 ·2	**18 ·02**	0 ·02261
(3) *Helianthus annuus.* 23/8/99. Intermittent sunshine. Leaf detached .... (Carried on simultaneously with (3) of Table I)	22° ·6	303 ·9	10 ·4	4 ·0	218 ·9	**18 ·00**	0 ·02257
(4) *Helianthus annuus.* 25/8/99. In sunshine under canvas screen (Carried on simultaneously with (4) of Table I)	28° ·2	312 ·7	9 ·95	4 ·0	205 ·1	**16 ·39**	0 ·02056
(5) *Helianthus annuus.* 29/8/99. Detached leaf. Cloudy day...... (Carried on simultaneously with (5) of Table I)	19° ·3	411 ·2	16 ·4	3 ·25	243 ·2	**18 ·15**	0 ·02277
(6) *Catalpa bignonioides.* Detached leaf. Weather cloudy .... (Carried on simultaneously with (16) of Table I)	20° ·0	433 ·6	12 ·65	4 ·0	172 ·5	**9 ·94**	0 ·01247

The rate of assimilation of leaves in air which has been artificially enriched with carbon dioxide is seen to be considerably greater than it is in normal air. In a previous communication direct proof has been given * that up to five or six times the normal amount of CO_2, assimilation, *cœteris paribus,* is approximately proportional to the amount of that gas present. A further general proof of this proposition may be obtained by reducing the values given in the last column of Table II in the proportion of the amounts of carbon dioxide in the leaf-cases (as given by Column (3)) to that of average air of from 2·8 to 3 parts per 10,000. A series of values is then obtained of the same order of magnitude as those of the last column of Table I.

Section (2).—*A Comparison of the Direct Method of Determining the Rate of Assimilation in Ordinary Air with the Weighing Method of Sachs.*

Up to about twenty years ago the only known methods for determining the rate of assimilation under free air conditions were based on an estimate, more or less exact, of the increase in dry weight of a plant during a considerable period of its growth. In 1884, Sachs, in his classical paper "Ein Beitrag zur Kenntniss der Ernährungsthätigkeit der Blätter," † asserted that with due precautions the varying dry weights of known areas of leaf-lamina could be used as measures of the amount of carbohydrate produced in the leaf.

In the case of a detached leaf of *Helianthus annuus* he found that there was an increase in weight during several hours of favourable isolation corresponding to 0·01648 gramme per square decimetre of leaf area per hour. Under similar conditions detached leaves gained more in weight than leaves still attached to the plant, a result which Sachs attributed to the simultaneous depletion of the leaf in the latter instance, owing to the migration of some of the products of assimilation into the stem of the plant. By adding the rate of *loss* during the hours of darkness to the rate of *gain* during insolation, Sachs obtained certain values which he believed to represent approximately the rate of assimilation during the day time. For *Helianthus* and *Cucurbita* his final results are given below. For purposes of comparison with our own results we have calculated the corresponding amounts of carbon dioxide equivalent to the carbohydrate which this increase is supposed to indicate.

---

* 'Roy. Soc. Proc.,' vol. 70, p. 399.

† 'Arbeit. d. Bot. Instit. Würzburg,' vol. 3, p. 23.

Table III.—Total Assimilation per Square Decimetre of Leaf per Hour, according to Sachs.

	Grammes increase per square decimetre per hour.	Equivalent of CO_2 in cubic centimetres.
Helianthus annuus …………	0·01882	15·00
Cucurbita Pepo ………………	0·01502	11·97

In repeating these experiments in 1892, Brown and Morris* obtained the following results by the Sachs method, in which no correction is made for the assumed simultaneous depletion in the case of the leaves still attached to the plant.

Table IV.

	Grammes increase per square decimetre per hour.	Equivalent of CO_2 in cubic centimetres.
Expt. I. *Helianthus annuus*—		
A. Detached leaf …………	0·00985	7·85
B. Still attached to plant..	0·00460	3·66
Expt. II. *Helianthus annuus*—		
A. Detached leaf …………	0·0100	7·97
B. Still attached to plant..	0·0071	5·66

When we compare the results of Table I obtained for *Helianthus* by direct determination of the carbon dioxide taken in by the leaf with those deduced by Sachs from his weighing method, we find a very great discrepancy, the apparent rate of assimilation in the former case being only one-third to one-fifth of what it is in the latter. On the other hand, in the experiments of Brown and Morris, where diurnal depletion of the leaf is not assumed, the discrepancy is less, but even here, in the case of the detached leaves, the weighing method has given an apparent rate of assimilation about double that of the direct method by CO_2 measurement.

In considering the probable cause of these discrepancies we must first discuss how far Sachs was justified in adding the hourly *gain* of weight of the leaf during the day to the hourly *loss* during the night in order to arrive at his result. He was led to this correction by observing that in placing two leaves under similar external conditions, but having one detached with its petiole in water, whilst the other was still attached to the plant, the gain in weight of the detached leaf per unit area was greater than that of the attached leaf. Whilst, for instance, the detached leaf gained in weight at the rate of 0·01648 gramme per decimetre per hour, the leaf on the plant only gained at the rate of 0·00914 for the same area and time, thus showing a falling-off of 44·5 per cent.

---

* 'Journ. Chem. Soc.,' 1893, Trans., p. 625.

The experiments of Brown and Morris also showed the same falling off in weight-increase in the case of the attached as compared with the detached leaves as shown in Table IV, this falling-off being 53 per cent. in Experiment (1) and 30 per cent. in Experiment (2), or an average of 41·5 per cent., *i.e.*, almost exactly Sachs' value.

There can be no doubt about the different behaviour of attached and detached leaves under the above conditions, but we believe the fact has received an erroneous explanation which has led to a considerable over-estimate of the rate of assimilation as deduced from the Sachs weighing method.

When the rate of assimilation of two similar leaves placed side by side is determined by the actual amount of carbon dioxide taken into the leaf, we have found that, contrary to what might have been expected, the leaf attached to the plant assimilates at a *less* rate than the detached leaf with its petiole in water. This is shown by the two experiments following, exactly similar leaves being enclosed in similar leaf-cases and exposed under identical conditions of insolation and air-current.

The falling-off in the assimilation of the attached leaf as compared with the detached leaf is 34·3 per cent. in Experiment (1) and 54·8 per cent. in Experiment (2), the mean being 44·5 per cent., which corresponds almost exactly with the differences in weight of the attached and detached leaves of *Helianthus* in the previously-recorded experiments of Sachs. But in this instance we cannot fall back upon leaf depletion of the attached leaf as the explanation, since our method, unlike that of Sachs' is quite independent of any migration of the products of assimilation during the experiment, for it is based on the actual intake of carbon dioxide into the leaf. We are forced to conclude therefore that the differences recorded in Table V are due to *differences in the stomatal openings* in the two cases, the stomata of the detached leaves being more widely opened than those of the leaf still attached to the plant.

Table V.—Relative Rate of Assimilation of *attached* and *detached* Leaves of *Catalpa bignonioides*, determined by Estimation of CO_2 absorbed from ordinary Air.

Plant.	Temperature, C.	Area of leaf in square centimetres.	CO_2 in air, parts per 10,000.		Duration of experiment in hours.	CO_2 absorbed during experiment in cubic centimetres, N.T.P.	CO_2 assimilated per square decimetre of leaf per hour.	Carbohydrate assimilated per square decimetre per hour, in grammes.
			Mean CO_2 of air in leaf-case.	CO_2 in outer air.				
(1) *Catalpa bignonioides.*								
8/8/99. A. Detached leaf ...	—	284 9	2·268	2·80	5·00	35·23	**3·053**	0·00382
B. Attached leaf ...		384·2	2·295	2·80	5·00	31·56	**2·003**	0·00251
(2) *Catalpa bignonioides.*								
15/8/99. A. Detached leaf ...		446·9	2.496	2·80	4·33	29·96	**1·735**	0·00217
B. Attached leaf ...		463·4	2·660	2·80	4·33	14·95	**0·784**	0·00098

From the physical considerations adduced in a previous paper it has been shown that gaseous diffusion through stomata must vary, other things being equal, with the linear dimensions of the openings, and it appears to be a fair deduction that the linear dimensions of the stomata in a leaf still attached to the plant are about 45 per cent. less than those of the stomata of the same leaf after separation from the plant and immersion of the petiole in water.

That the artificial conditions under which such a detached leaf is placed should have an influence on the delicate self-regulating mechanism of the guard-cells of the stomata is perhaps scarcely to be wondered at, especially when we bear in mind that a leaf which is still attached to its plant is receiving water more or less highly charged with salts derived from the soil, which must exert a distinct osmotic effect on the leaf-cells, whereas only pure water is supplied to the cut leaf.

Whether or not this is the true explanation of the observed differences the fact remains that for equal areas, and under similar conditions, the rate of assimilation of a leaf still attached to the plant is about 45 per cent. less than that of a detached leaf, and this must in future be taken into consideration in interpreting the experiments of Sachs, in which equality in assimilatory power in the attached and detached leaves has hitherto been assumed.

It would appear therefore that Sachs was not justified in making the correction for diurnal depletion which he did; but even if we neglect this supposed depletion and assume that none of the products migrate from the leaf to the stem during the insolation, the apparent increase in weight of an attached leaf, both in the experiments of Sachs and those of Brown and Morris, still indicates a rate of assimilation about twice that indicated by the direct measurement of the intake of carbon dioxide, as given in Table I.

The first explanation of the discrepancy which suggests itself is that the conditions for assimilation in our leaf-cases are not so favourable as those which exist when the leaf is freely exposed to the air. We have already seen that when cut leaves are employed the advantage in one respect (provisionally ascribed to the wider opening of the stomata) is in the direction of obtaining more rapid photosynthesis than in leaves attached to the plant. There is another aspect of the subject, however, which requires careful consideration. Owing to the practical difficulty of keeping down the temperature of the leaf enclosed in its case it is impossible to carry out such experiments successfully in full sunshine, and they must be made either on cloudy days, or by moderating the sunshine by an artificial "cloud" in the nature of a thin canvas screen. Unless it can be shown that in this moderated illumination the particular rays active in the photosynthetic process are still considerably in excess of the requirements of the chloroplasts for the particular partial pressure of the carbon dioxide employed, there will be room for doubting whether in this respect the leaf enclosed in its case is not at a disadvantage as compared with a leaf receiving the full solar radiation under free-air conditions.

We have made numerous experiments in two different directions in order to throw some light on this point. In the first place we find that under this moderated illumination, whether the diminution of intensity is due to natural cloud or to the interposition of a thin canvas screen, the assimilatory process always readily responds to slight increases in the amount of the carbon dioxide in the air of the case, and that within certain limits fixation of carbon dioxide is proportional, or nearly so, to the partial pressure of that gas. This fact in itself indicates that the special rays active in photosynthesis are still present in the moderated illumination in excess of the requirements of the leaf when it is in air of normal CO_2 content.

Another, and still more conclusive mode of experiment was to place two similar leaves in their cases under the thin canvas screen, cutting off a certain determinate amount of the light from one of them by means of the apparatus with revolving sectors, already described in Part I, and measuring the relative rate of assimilation by estimating the carbon dioxide absorbed from a stream of ordinary air passed through the cases.

The results of such a series of experiments are recorded in the following Table VI. It will be seen that the sunlight passing through the light canvas screen, which absorbed about two-thirds of the solar radiation, had to be further reduced by the radial sectors to *one-quarter* of its intensity before there was any sensible effect in reducing the rate of assimilation ; thus showing that the light which passed through the screen still contained more of the particular grade of radiant energy active in photosynthesis than the chloroplasts of the leaf could utilise. This appears to us to be a strong argument in favour of the leaves enclosed in the cases being under no disadvantage as compared with those under free-air conditions. In fact, for reasons already given, it would appear that we might expect better results from the *cut* leaves when placed under these artificial conditions than are attainable with leaves still attached to the plant in the open.

Table VI.—Influence of Reduced Illumination of Leaves of *Tropæolum majus* on Rate of Assimilation. Ordinary air used.

A. Leaves exposed to sunlight which had passed through thin canvas screen.
B. Leaves also under canvas screen, but light still further reduced by revolving sectors.

N.B.—The volumes of carbon dioxide absorbed by the leaves are corrected to the partial pressure of that gas in outer air.

	Temperature in leaf-case.	CO_2 assimilated by leaf per decimetre per hour in cubic centimetres (corrected).	Carbohydrate corresponding to CO_2 in grammes.
Experiment (1).—			
4/9/00. A. Full illumination (under screen)	21°·7	1·692	0·00212
B. Half illumination (under screen)	20°·3	2·029	0·00254
Experiment (2).—			
18/9/00. A. Full illumination (under screen)	20°·6	1·919	0·00240
B. Half illumination (under screen)	19°·6	2·047	0·00256
Experiment (3).—			
13/9/00. A. Full illumination (under screen)	24°·2	1·926	0·00241
B. Quarter illumination (under screen)	22°·2	1·311	0·00164

The following Table VII gives the results of a further series of experiments in which the rate of assimilation of detached leaves under free-air conditions was deduced from the ordinary half-leaf weighing method of Sachs, species of plants being taken in which the leaf-symmetry is especially well marked. The individual leaves were selected with great care to ensure as close a correspondence as possible between the two halves.

Table VII.—Rate of Assimilation deduced from Half-leaf weighing Method.

Species of plant.	Time of insolation in hours.	Area in square centimetres.	Dry weight in grammes.	Increase in dry weight per square decimetre per hour, in grammes.
(1) 3/8/98.— *Catalpa bignonioides*—				
A. Before insolation......	—	259·1	1·1188	—
B. After insolation	7·0	276·8	1·3488	0·00791
(2) 9/8/98.— *Catalpa bignonioides*—				
A. Before insolation......	—	225·6	0·9729	—
B. After insolation	5·0	194·1	0·9740	0·01370
(3) 16/8/98.— *Catalpa bignonioides*—				
A. Before insolation......	—	418·35	1·8546	—
B. After insolation	3·25	438·45	2·1812	0·01660
(4) 2/6/98.— *Sparmannia africana*—				
A. Before insolation......	—	150·0	0·6303	—
B. After insolation	6·25	150·0	0·6636	0·00387
(5) 3/6/98.— *Sparmannia africana*—				
A. Before insolation......	—	125·0	0·5086	—
B. After insolation	6·0	125·0	0·5287	0·00268
(6) 22/6/98.— *Sparmannia africana*—				
A. Before insolation......	—	125·0	0·4295	—
B. After insolation	6·5	125·0	0·4734	0·00540

N.B.—In Experiments (4), (5), and (6) equal areas were cut from the two halves of the leaves before and after insolation. In the other experiments the entire half leaves were taken.

In the results recorded up to this point we have seen that the general tendency of the Sachs' weighing method is to give a much higher estimate of the rate of assimilation of leaves in ordinary air than is given by a method based on the measurement of the actual intake of carbon dioxide. The justice of this comparison of methods rests so far on the assumption that the leaves enclosed in their cases are under conditions which, at any rate, are not *less* favourable to assimilation than those experienced by leaves assimilating in free air. The reasons in favour of the correctness of this assumption have been already given, but in order to meet any possible objections on this point we instituted another series of experiments *in which the increase in dry weight and the simultaneous intake of carbon dioxide could be determined on the same leaves.*

These experiments were made on leaves of *Catalpa bignonioides* in the following manner. Four selected symmetrical leaves, whilst still attached to the plant, were covered with tin-foil in the evening in order to ensure their effective depletion by the morning of the following day. These leaves were then carefully detached by cutting through the petioles under water, and were brought into the laboratory. From each pair of leaves the alternate right and left halves were then cut away along the mid-rib, and the halves still attached to the mid-rib were arranged in a large glazed experimental case of the usual pattern, the petioles of each pair of halves dipping into test-tubes of water fixed outside the case. These halves were then placed in strong light of sufficient intensity to cause the leaves to assimilate freely, and a fairly rapid stream of air was drawn through the case, the carbon dioxide taken in by the leaves being estimated in the usual manner.

At the close of the experiment, which lasted some hours, the half-leaves were removed from the case and the mid-ribs were sliced off, the respective areas of the separated laminæ and mid-ribs being determined separately by the application of the planimeter to the photographic prints. From the total amount of carbon dioxide assimilated during the experiment a proportional deduction was made for the area of the mid-rib portions which had taken part in the assimilation. The dry weight of the separated laminæ was then determined, the final drying taking place in a current of dry hydrogen at 100° C.

Meanwhile, the area and dry weight of the other halves separated at the commencement of the experiment were determined, when we had all the data for determining (1) the increased weight of the leaf-lamina per square decimetre per hour (Sachs' method), and (2) the intake of carbon dioxide and the corresponding amount of carbohydrate produced per square decimetre per hour. The final results of four such experiments are given in the following Table VIII. The areas of leaf employed varied from about 550 to 750 sq. cm.

Table VIII.—Direct Comparison of the Increase in Weight during Insolation of the Leaf-Lamina of *Catalpa bignonioides*, with the Intake of CO_2 from ordinary Air, and the corresponding Amount of Carbohydrate formed.

	Increased weight of leaf-lamina per square decimetre per hour in grammes.	CO_2 absorbed by leaf per square decimetre per hour in cubic centimetres.	Carbohydrate formed per square decimetre per hour deduced from CO_2 absorbed, in grammes.
Experiment (1).........	0·00983	1·41	0·00176
„ (2).........	0·00714	1·43	0·00179
„ (3).........	0·00260	2·35	0·00294
„ (4)........	0·00722	2·33	0·00292
Mean	0·00669		0·00235

A comparison of the first and third columns in the above table shows a very considerable discrepancy between these two methods of estimating the rate of assimilation.

In only one case, that of Experiment (3), is there any kind of agreement between the results of the weighing method and that based on intake of carbon dioxide: in all other instances the Sachs' method gives results which are far in excess of the direct method. If we take the mean of all four experiments we find that the Sachs' method gives an estimate of the assimilation rate between two and three times greater than that deduced from the intake of carbon dioxide, a result which agrees fairly well with the previous experiments which were not carried out under conditions admitting of such rigorous comparison as those of Table VIII.

The discrepancy is far too great to be accounted for by any under-estimate of the amount of carbohydrate equivalent to the fixation of a given amount of carbon dioxide, and can only be explained by some error incident to the Sachs' method of determination. This is the more certain since in one experiment, the results of which are not recorded in Table VIII, the apparent dry weight of the leaf-lamina per unit-area was actually *less* after insolation of the leaf than it was before, although there had been an intake of carbon dioxide corresponding to the assimilation of 0·003 gramme per square decimetre per hour.

The great objection to the Sachs' method is that all the various errors to which it is liable *are accumulative in the result,* and affect that result absolutely. Besides the ordinary errors to which operations of measuring and weighing are liable, and which may be so far reduced as to be almost negligible, there are three possible sources of error which may have a very serious influence on the result. In the first place it is by no means certain that the cell-wells and contents of the leaf-cells after insolation are in the same condition as regards their constitutional water as they were before insolation. We are not referring here to such metabolic changes as those due to the hydrolysis of starch or cane-sugar or the reverse changes, for although such transformations undoubtedly result in the fixation or liberation of water, and theoretically must have an effect on the final dry weight of the leaf, yet it can be shown that this effect must be relatively small. We refer rather to possible changes in the power of retention of water at 100° C. by the colloidal elements of the cell-contents. A very small change in this power of water retention might make a very appreciable difference in the final estimate by the Sachs' method of the apparent amount of matter assimilated; but the two principal sources of error in the Sachs' method, compared with which all others sink into comparative insignificance, are no doubt (1) differences due to want of perfect symmetry in the venation and the thickness of the lamina, and (2) alterations of area in corresponding parts of the leaf, one of which is measured at once, and the other only after being placed in the light for some hours, during which time, although the leaf may apparently remain unaltered, it is put under a different state of tension.

We have made a number of experiments on both these points. In the first place, in investigating the degree of symmetry which exists on the two sides of a leaf, only those leaves were taken which appeared to the eye to be quite symmetrical. The two sides of the leaf were then separated carefully from the mid-rib, printed on photographic paper, and the area of each approximately equal side carefully taken with the planimeter. We may here mention that errors of planimeter measurement fall well within 0·1 per cent.

The halves of the leaves were then dried under exactly the same conditions in a slow current of dry air until constant in weight.

Another series of experiments was then made in order to estimate the possible difference in area, due to *shrinkage or the reverse,* which a leaf undergoes on insolation. A leaf of *Catalpa bignonioides* was divided down the middle, and the half to which the petiole was still attached was printed on photographic paper, and the area determined with the planimeter. These half-leaves were then placed in a glazed assimilation-case and exposed to sunlight under a canvas screen, air being drawn through the case just

Table IX.—Test of Symmetry between Opposite Sides of Leaves.

R. = Right half. L. = Left half.

Name of plant.	Area in square centimetres.	Dry weight in grammes.	Dry weight per square decimetre in grammes.	Difference, per cent.
Catalpa bignonioides......	R. 114·32	0·4652	0·4069	
	L. 99·90	0·3906	0·3909	−3·9
,, ,, ...	R. 164·6	0·8482	0·5153	
	L. 155·8	0·7680	0·4929	−4·3
,, ,, ...	R. 126·75	0·6024	0·4752	
	L. 131·27	0·6386	0·4864	+2·3
,, ,, ...	R. 111·95	0·4211	0·3761	
(This leaf had been depleted by covering)	L. 103·90	0·3685	0·3546	−5·7
Catalpa bignonioides......	R. 107·3	0·4983	0·4644	
	L. 108·32	0·4994	0·4610	−0·7
Catalpa purpurea	R. 89·1	0·4526	0·5079	
	L. 90·6	0·4716	0·5184	+2·0
Catalpa Bungei............	R. 112·6	0·6261	0·5560	
	L. 114·8	0·6472	0·5637	+1·3
,, ,, ...	R. 110·5	0·6504	0·5855	
	L. 96·2	0·5533	0·5751	−1·7
Tropæolum majus	R. 47·00	0·1754	0·3732	
	L. 43·35	0·1624	0·3746	+0·3
Polygonum Weyrichii ...	R. 173·1	0·9124	0·5270	
	L. 177·6	0·9252	0·5209	−0·1
			Mean error...	±2·2

Table X.—Influence of Exposure of Leaves in altering Area.

Half leaf.	Area before insolation in square centimetres.	Area after insolation in square centimetres.	Difference per cent. induced by insolation.
(1) *Catalpa bignonioides*	112·2	108·7	−3·12
(2) ,, ,, ...	111·9	113·0	+0·98
(3) ,, ,, ...	138·4	138·9	+0·36
(4) ,, ,, ...	137·1	137·3	+0·14
		Mean......	±1·1

as in experiments on assimilation. After six hours' exposure the leaves were again printed, and the areas determined afresh.

It will be seen on examining Tables IX and X that the errors which may be introduced into the Sachs' weighing method from the causes there noted may be of considerable magnitude. The average error observed due to differences in symmetry amount to 2·2 per cent. and that due to change of area to 1·1 per cent. Assuming that the accumulated errors from all sources in a Sachs' experiment amounted to 2·0 per cent., with a leaf having a dry weight of 0·50 gramme per square decimetre, this would lead to an over or under-estimate of the matter assimilated of 0·010 gramme per square decimetre for the total time of experiment, and if the duration of the experiment were five hours, to an apparent assimilation or depletion at the rate of 0·002 gramme per decimetre of leaf per hour. But this is about the average amount of true assimilation observed for the leaf of *Catalpa bignonioides* by the direct method of determining the carbon dioxide assimilated, so that an under-estimate of the area of only 2 per cent. in the insolated half-leaves would on the Sachs' weighing method give an over-estimate of the assimilation of 100 per cent. of the true value, whereas it would only affect the results obtained from our method of carbon dioxide absorption to the extent of 2 per cent.

In the light of these experiments we cannot avoid the conclusion that the Sachs' method cannot be trusted for anything like exact quantitative estimation of the photosynthetic work which is going on in an assimilating leaf. As ordinarily applied its general tendency is to give far too high an estimate of the rate of assimilation, which can only be measured with any approach to exactness by a determination of the actual intake of carbon dioxide into the leaf from an atmosphere containing that gas in small and determinate amounts.

Section (3).—*The Relation of the Distribution of Stomata to the Rate of Gaseous Exchange in the Leaf.*

This is a subject which has been very fully and satisfactorily investigated by F. F. Blackman,* who demonstrated for the first time that the exchange of carbon dioxide between the leaf and the surrounding air closely follows the stomatic distribution on the two surfaces of the leaf, and that the experiments can only be interpreted on the supposition that the gaseous exchanges take place by free diffusion through the open stomates and not by diffusion across the imperforate portions of the leaf-cuticle.

---

* 'Phil. Trans.,' vol. 186 (1895), p. 502.

As regards the exhalation of the carbon dioxide of respiration, Blackman's experiments are quite conclusive, but his method does not appear to be quite so well adapted to investigations of the assimilatory intake where, owing to the restrictions of time and leaf area, the amounts of carbon dioxide which have to be dealt with seldom exceed about 0·1 c.c., with a possible experimental error of about one-tenth of this amount, and where the conditions of experiment are such as to require "much watchfulness, and a favourable concurrence of circumstances such as is rarely accorded."

Since the method which we have employed for determining the rate of assimilation in ordinary air is one which readily lends itself to investigations of this nature, we have considered it desirable to repeat these classical experiments of Blackman under conditions which admit of measuring the intake of relatively large amounts of carbon dioxide from ordinary air by the two sides of a leaf on which the distribution of stomata is known.

For this purpose special leaf-cases were constructed which were similar in principle to those described by Blackman.* They consisted of a pair of shallow Petri-dishes fixed into light brass frames with flanges which could be pressed together by simple spring-clips. When the leaf was clipped between the two flanges and the junction was made tight with soft wax the lamina formed a diaphragm dividing the case into two compartments, one connected with the upper and the other with the lower side of the leaf. Through each of these compartments a separate current of air could be drawn by means of small brass tubes fitted into the sides of the chambers.

Two of these cases were used, one exposing a leaf-area of 28·27 sq. cm., and the other 59·44 sq. cm.

Measured volumes of ordinary air of known carbon dioxide content were aspirated through the cases in the way already described at rates varying from 10 to 20 litres per hour, the absorption and determination of the carbon dioxide in the emergent air being effected as usual.

The number of stomata on known areas of the upper and lower sides of the leaf was, in all cases determined under the microscope by actual counting, the mean of a large number of observations being taken.

In order to make the comparison as strict a one as possible the results of each pair of experiments have been reduced to equal partial pressures of carbon dioxide in the air passing through the case. Such a correction is very necessary in experiments on respiration in which there is sometimes a considerable difference in the mean composition of the air in the two compartments.

* *Loc. cit.*, p. 521.

Table XI.—Respiration of Amphistomatous Leaves showing Relation of Carbon Dioxide evolved by Upper and Lower Surface, and Ratio of Distribution of Stomata.

U. = Upper surface. L. = Lower surface.

Plant.	Time in hours.	Leaf area in square centimetres.	CO_2 evolved in cubic centimetres.	Ratio of CO_2 evolved. $\frac{\text{Upper}}{\text{Lower}}$.	Ratio of stomatic distribution. $\frac{\text{Upper}}{\text{Lower}}$.
(1) *Canna indica*...	4·75	28·27	U. 8·41 L. 20·76	$\frac{100}{246}$	$\frac{100}{246}$
(2) " " ...	5·0	28·27	U. 5·55 L. 17·90	$\frac{100}{322}$	$\frac{100}{246}$
(3) " " ...	4·23	28·27	U. 3·04 L. 6·40	$\frac{100}{210}$	$\frac{100}{246}$
(4) *Rumex alpinum*	5·5	59·44	U. 1·03 L. 3·60	$\frac{100}{286}$	$\frac{100}{269}$

Table XII.—Assimilation of Amphistomatous Leaves illuminated on Upper Surface, showing Relation of Intake of Carbon Dioxide by the Upper and Lower Sides and the Ratio of Distribution of Stomata.

U. = Upper side. L. = Lower side.

Plant.	Time in hours.	Leaf area in square centimetres.	CO_2 assimilated, in cubic centimetres.	Ratio of CO_2 assimilated. $\frac{\text{Upper.}}{\text{Lower.}}$	Ratio of stomatic distribution. $\frac{\text{Upper.}}{\text{Lower.}}$
(1) *Colchicum speciosum* ...	5·75	59·44	U. 4·34 L. 3·26	$\frac{100}{72}$	$\frac{100}{119}$
(2) *Senecio macrophyllum*...	4·75	28·27	U. 3·90 L. 3·60	$\frac{100}{92}$	$\frac{100}{126}$
(3) " " ...	4·25	28·27	U. 5·80 L. 4·20	$\frac{100}{72}$	$\frac{100}{126}$
(4) *Rumex alpinum*	5·0	59·44	U. 5·70 L. 8·90	$\frac{100}{144}$	$\frac{100}{269}$
(5) " "	5·5	59·44	U. 7·50 L. 9·81	$\frac{100}{130}$	$\frac{100}{269}$

In the following Table XIII are recorded the results of two assimilatory experiments on leaves which have their stomata on one side only. In *Nuphar* the stomates are on the upper, and in *Catalpa* on the lower side of the leaf.

Table XIII.

Plant.	Time in hours.	Area in square centimetres.	CO_2 assimilated, in cubic centimetres.
(1) *Nuphar advena* (hyperstomatous)	2·0	76·97	U. 2·20 L. 0·00
(2) *Catalpa bignonioides* (hypostomatous)	1·85	79·03	U. 0·00 L. 4·91
(3) ,, ,, ,,	2·3	79·03	U. 0·00 L. 8·96

The results fully confirm in most respects the observations of Blackman, and may be summarized as follows:—

(1). In the *respiration* of amphistomatous leaves (*e.g.*, *Canna indica* and *Rumex alpinum,* see Table XI) the ratio of the carbon dioxide evolved from the upper and lower surface of the leaf follows very closely the ratio of the distribution of stomates.

(2). In the *assimilatory process* of amphistomatous leaves illuminated on the upper surface (see Table XIII), the intake of carbon dioxide by the lower surface is always *less* than might be expected from the relative number of stomata on the two sides, the amount of carbon dioxide assimilated by the under side in some cases falling to half that deduced from a consideration of the stomatic distribution.

(3). If the leaf is hypostomatous (vide *Catalpa,* Table XIII), the intake of carbon dioxide during assimilation only takes place on the *lower* (stomatiferous) surface.

(4). When the leaf is hyperstomatous (vide *Nuphar,* Table XIII), the intake of carbon dioxide is only on the upper (stomatiferous) surface.

That there should be a much closer correspondence between the ratios of stomatic distribution and the ratios of gaseous exchange in the respiratory than in the assimilatory process, follows from what we know of the physics of diffusion through fine apertures.

Assuming that there is a steady evolution of carbon dioxide going on in the leaf-cells during respiration, the rate at which this will diffuse into the surrounding air will be quite independent of the degree of opening of the stomata. If the stomata partially close whilst this outward diffusion is going on, the partial pressure of the carbon dioxide within the leaf will increase, and this increase will be inversely proportional to the altered linear dimensions of the opening. This rise in "diffusion potential" will, consequently, exactly counterbalance the effect of the diminished size of the stomatic opening, and *the same amount of respiratory carbon dioxide will consequently escape from the leaf, no matter what changes in the size of the opening takes place,* provided these are not sufficient to close the stomata completely.

This however does not apply to the intake of atmospheric carbon dioxide into the leaf during the assimilatory process. Providing a sufficient amount of the right grade of energy is reaching the chloroplasts, the "diffusion potential" in this case will remain constant, the partial pressure of the inward diffusing carbon dioxide varying from 3/10,000 of an atmosphere outside the leaf to zero at the point where complete absorption takes place. Under these conditions the intake of carbon dioxide during assimilation must vary directly with the linear dimensions of the openings. Hence in an amphistomatous leaf the relative rate of assimilation by the two sides will not only be influenced by the number of stomata on equal areas in each case, but also on the degree to which these stomata are opened, a condition which, as we have seen, is not operative in the gaseous exchange of carbon dioxide in respiration.

Partial opening of the stomata on the upper side is extremely likely to be brought about when the incidence of illumination and radiant energy is on that side of the leaf, and in such a case we might expect exactly the results which are recorded in Table XII. There is, however, another factor which no doubt contributes to the apparent excess of assimilation by the upper surface of such leaves. By far the greater part of the particular grade of radiant energy which produces photosynthesis is doubtless absorbed by the chloroplasts of the palisade parenchyma into which the stomata of the upper side of the leaf open. The diffusion gradient will therefore be "steeper" in the intercellular spaces of the palisade parenchyma than in those of the more deeply seated spongy parenchyma, a fact which will in itself favour a more rapid inflow of carbon dioxide into the leaf through the stomata of the upper side.

Section (4).—(*a*) *Experiments on Leaves Exposed to Artificial Illumination in a Current of Ordinary Air.*

These experiments were made during the winter months of 1900–1901 with cut leaves of greenhouse-plants illuminated on the upper sides with light from a large No. 4 Welsbach burner.

They were instituted with the idea of working out the influence of varying external conditions on the assimilatory process under constant illumination, but failed in this object owing to the comparatively feeble photosynthetic power of the source of light used, and the abnormal respiration which occurs at this time of the year in greenhouse-plants. These two causes resulted in a complete masking of the assimilatory process as measured in the ordinary way, the air which had been in contact with the leaf always gaining in carbon dioxide owing to respiration being in excess of assimilation.

More satisfactory results would, no doubt, have been obtained by this method in the summer, but this line of research was abandoned when it was found that definite variations in the intensity of sunlight or diffused daylight could be conveniently produced by the revolving radial sectors already described, thus admitting of comparisons being made in simultaneous experiments lasting for some hours, although the actual intensity of the unobstructed radiation might vary considerably during that time.

Nevertheless some of these experiments with artificial illumination are not devoid of interest, and we have, therefore, briefly alluded to a few of them.

The actual heating effect* of a large Welsbach burner placed within a foot or two of a leaf enclosed in its glazed case is considerable, and had to be neutralised as far as possible by passing the light through a water-cell 2 or 3 inches in thickness. Where comparisons were made with the respiration effect in darkness, the respiratory chamber was as far as possible brought to the same temperature as the one in which the assimilatory effect was investigated.

The leaf was enclosed as usual in its case through which a measured stream of air was aspirated, of which the carbon dioxide-content was determined at ingress and egress, and the case was suitably arranged so that no light could reach the leaf except that coming from the gas burner.

The temperature of the air in the case was given by a thermometer inserted through the side, and since a fairly rapid stream of air was passing through the apparatus, and the leaf was under favourable conditions for transpiration, it is almost certain that the temperature of the leaf cannot have been far removed from that of the air surrounding it.

---

* The total radiation from the Welsbach burner employed, at a distance of 1 foot and with only a thin glass screen, was found by the Callendar's radiometer to be 0·089 cal. per sq. cm. per minute. When the water-cell was interposed this fell to 0·031 cal. per sq. cm. per minute. At a distance of 2 feet these values were, of course, reduced to one quarter.

Table XIV.—Experiments on Leaves illuminated by Welsbach Light.

Name of plant.	Distance of source of light from leaf in feet.	Temperature, C.	Area of leaf in square centimetres.	Duration of experiment in hours.	CO_2 evolved during experiment in cubic centimetres.	CO_2 evolved per square decimetre per hour in cubic centimetres.
December 25— *Dioscorea cayennensis.*						
(1) A. In darkness...	—	19°·1	200·0	2·2	40·30	9·16
B. Illuminated...	1	22°·3	—	2·3	33·58	7·30
January 10— *Dioscorea cayennensis.*						
(2) A. In darkness...	—	19°·9	169·4	2·8	36·35	7·662
B. Illuminated...	1	22°·7	—	2·55	22·17	5·129

Partial re-assimilation of the carbon dioxide of respiration has evidently taken place in both instances under the influence of the artificial illumination, in (1) to the extent of about 1·86 c.c. per sq. decimetre per hour, and in (2) to the extent of about 2·53 c.c. These it is true are amounts which compare favourably with the rate of assimilation of leaves exposed to sunlight in ordinary air containing the normal amount of carbon dioxide, but we are certainly not justified in deducing that the Welsbach light is rich in photosynthesizing rays, since the conditions are widely different. In ordinary air the maximal partial pressure of the carbon dioxide surrounding the assimilating centres must fall considerably short of 3/10,000 of an atmosphere, whereas during active respiration the air of the intercellular spaces may be highly charged with that gas, thus increasing the assimilatory effect in proportion. In the former case the limiting factor is the small partial pressure of the carbon dioxide, the photosynthetic rays being in excess; whilst in the latter case the limitation is due to the comparative want of a sufficient amount of radiant energy of the right kind, and not to paucity of carbon dioxide.

That this is the true explanation was shown by another experiment on one of these very actively respiring leaves of *Dioscorea* by illuminating it in the laboratory with feeble winter sunlight, when the amount of carbon dioxide evolved fell to 0·28 c.c. per square decimetre per hour.

(*b*) *Experiments on Respiration.*

The abnormal respiratory effect observed in greenhouse plants during the winter months is shown in the following Table XV, and in Table XVI we have shown by way of contrast the normal respiration observed in certain leaves during the *summer*, the plants in this instance having been grown under ordinary out-door conditions.

Table XV.—Winter Respiration of the Leaves of Greenhouse-plants.

Date.	Name of plant.	Temperature, C.	CO_2 respired per square decimetre per hour in cubic centimetres.
December 14.........	*Senecio grandifolius*	25°·2	5·12
,, 20.........	*Dioscorea cayennensis*	22°·7	13·45
,, 26.........	,, ,,	19°·0	8·78
,, 5.........	,, ,,	19°·9	5·33
,, 12.........	*Plumeria lutea*	19°·7	2·49
,, 7.........	*Begonia haageana* ...	16°·5	3·38
,, 13.........	*Sapium bolinense*......	18°·7	8·98

Table XVI.—Summer Respiration of Leaves grown under Out-door Conditions.

Date.	Name of plant.	Temperature, C.	CO_2 respired per square decimetre per hour in cubic centimetres.
August 22	*Helianthus annuus* ...	24°·1	0·429
September 7	,, ,, ...	26°·8	0·714
June 26	*Petasites albus*.........	18°·0	0·321
July 5	,, ,, ...	25°·6	0·584
June 22	*Polygonum Weyrichii*	17°·8	0·721
July 3	,, ,,	25°·2	0·788
July 11	,, ,,	36°·4	1·179

It will be noticed that, except in the last instance, where the temperature was very high in the respiration case, the respiration of these leaves growing under normal out-door conditions does not average more than 6/10 c.c. of carbon dioxide per square decimetre of leaf per hour, or less than one-tenth of the amount observed in the winter respiration of the greenhouse plants.

The influence of increased temperature in accelerating respiration is, as is well known, very considerable. This is illustrated in a general way by the following experiments made in the summer of 1900 on leaves of *Helianthus annuus* whilst still attached to the plant.

Table XVII.—Influence of Temperature on the Respiration of Leaves of *Helianthus annuus.*

Temperature, C.	CO_2 respired per square decimetre per hour in cubic centimetres.
19°·6	0·579
31°·2	1·656
36°·3	1·839
39°·2	2·080
41°·7	2·451

The above experiments are not strictly comparable, since they were made on different leaves of the same plant and on different days. A more complete series was made in a darkened glass cylinder immersed in water which was kept at a constantly regulated temperature, but the record of this experiment has been lost.

PART III.—THE ENERGETICS OF THE LEAF.

CONTENTS.

PAGE
Section (1). General Considerations of the Thermal Relations of a Leaf to its Surroundings 69
Section (2). Experimental—
- (*a*) Determination of the Total Incident Radiation 88
- (*b*) The Absorption of Solar Radiation by the Leaf-lamina 90
- (*c*) The Selective Absorption of Radiant Energy by the Leaf 93
- (*d*) Relative Absorption of Solar Radiation by Albino and Green Leaves ... 94
- (*e*) The Thermal Emissivity of the Leaf-lamina 97
- (*f*) Details of Experiments on Leaves submitted to Solar Radiation, showing the Mode of Disposal of the Incident Energy under Defined Conditions 97

Section (1)—*General Considerations of the Thermal Relations of a Leaf to its surroundings.*

Before we can profitably discuss the general question of the exchange of energy between a living leaf and its environment, it is necessary to consider those physical and chemical changes occurring within the leaf which are attended by any sensible evolution or absorption of energy.

Of these there are only three which need be taken into serious account; (1) the vaporization of water; (2) the photosynthesis of carbohydrates; and (3) the chemical changes attendant on respiration.

The first two changes are *endothermic* in character, but are as a rule of very dissimilar value as regards the actual thermal disturbances induced, (1) under ordinary circumstances being very large compared with (2).

The respiratory changes (3) are *exothermic* in their final result.

By suitable means the loss or gain of heat due to each of the above-mentioned changes can be determined in water-gramme units, and referred to the total amount of energy received by the leaf from its surroundings, either through radiation or the convective and conductive action of the air. In order, however, to complete our knowledge of the thermal relations of leaf and environment, we must know something of the absorptive power of the leaf-lamina for the particular form of radiant energy incident upon it, and also its *thermal emissivity*, using that term in its wide sense to include the loss or gain of heat due to radiation and air-convection and conduction which unit-area of the leaf will experience in unit-time, with unit difference of temperature between itself and its surroundings. In some cases we also require to know the weight of unit-area of the lamina and its approximate specific heat. Our investigations, described in detail later on, have led us to fairly accurate methods for the determination of all these factors.

For some time the story could not be made complete, owing to difficulties in determining the *thermal emissivity* of a leaf. These difficulties have now been surmounted by the adoption of a comparatively simple method based on the differential self-cooling of leaves which are transpiring at unequal rates. The method is fully described in a separate communication.*

As a preliminary to the study of the thermal interchanges between a leaf and its surroundings, it will be convenient to consider two distinct sets of general conditions; (A) when the leaf is shaded from direct solar radiation or any other form of radiation which can produce photosynthesis; and (B) when the leaf is receiving solar radiation.

Case A.—*The Thermal Relations of a Leaf to its Surroundings when it is shielded from Solar Radiation.*

In order as far as possible to simplify the problem, we will assume in this case that a detached leaf, freely supplied with water, is placed in an enclosure, the walls of which are non-reflective and are maintained along with the enclosed air, at a perfectly uniform temperature which we will denote by θ. We will further assume that at the commencement of the experiment the leaf is also at this same temperature θ.

---

* See Brown and Wilson, *infra*, p. 122.

If the air of the enclosure is saturated with water-vapour, and there are no chemical changes going on in the leaf attended with evolution or absorption of energy, there will be complete thermal equilibrium in the system, all parts of which will remain at temperature θ.*

Since, however, active respiration is going on in the leaf-cells, this static thermal condition cannot be exactly maintained, and the leaf-lamina will consequently tend to rise slightly in temperature.

How small is the thermal disturbance due to this cause will be apparen from the following considerations.

The amount of carbon dioxide evolved is a measure of the oxidation of the leaf-substance used up in the respiratory process, hence, provided we know the nature of the oxidized substance, and its heat of combustion in absolute units, we can determine the liberated energy corresponding to the appearance of a given amount of carbon dioxide per unit-area of leaf per unit-time.

We may safely assume that the substance which is used up in respiration belongs to the class of carbohydrates. Direct evidence of this has been given by Brown and Morris,† who found that the disappearance of the sugars and starch of leaves of Tropœolum placed in the dark corresponded very closely with the loss of weight due to respiration.‡

The heat of combustion of the different carbohydrates for equal weights of contained carbon varies only within narrow limits, and we may therefore, without serious error, safely refer the carbon dioxide of leaf-respiration to a sugar of the *hexose* class, having a heat of combustion of about 3760 calories per gramme.

The actual amount of carbon dioxide respired by a leaf varies considerably with the temperature and with the age and species of the plant. At about 20° C., we have found healthy mature leaves of *Helianthus annuus* evolve about 0·70 c.c. of carbon dioxide per square decimetre of leaf-lamina per hour, or 0·000116 c.c. per square centimetre per minute. This corresponds to $1 \cdot 55 \times 10^{-7}$ gramme of dextrose, or to an evolution of heat of $1 \cdot 55 \times 10^{-7} \times 3760 = 0 \cdot 000582$ calorie per square centimetre of leaf-lamina per minute.

---

* It is here assumed that the partial pressure of the water-vapour in the interspaces of the leaf is in equilibrium with the vapour-pressure of a *plane surface* of water at the same temperature. Should it ever be proved that transpiration into a saturated atmosphere is really possible when the whole system is in perfect thermal equilibrium, the cause must be sought either in the increased vapour-pressure of minute convex surfaces, such as give rise to the distillation of small drops into large ones, or, in some such properties of colloids, as those suggested by the observations of J. M. van Bemmelen and v. Schröder, which indicate that a higher tension of water-vapour can be maintained over a "gel" than corresponds to a plane surface of water at the same temperature.

† 'Journ. Chem. Soc. Trans.,' 1893, p. 671.

‡ Compare also the results of T. C. Day ('Journ. Chem. Soc. Trans.,' 1880, p. 658), who showed that the ratio of the carbon dioxide and water liberated during the germination of barley corresponds closely with that required for the combustion of a glucose.

Since the weight of one square centimetre of the leaf-lamina of Helianthus is about 0·020 gramme, and its specific heat is about 0·879 (water = 1·0) the above amount of heat of respiration would be sufficient to raise the temperature of the leaf at the rate of 0°·033 C. per minute, *provided no simultaneous loss of heat were taking place by radiation, air-convection, or internal work of vaporization.*

But all these sources of loss of heat become operative directly the leaf temperature rises above θ, that of its surroundings, and the leaf can only come into thermal equilibrium with its surroundings at some temperature θ_n higher than θ, at which point the heat of respiration produced in unit-time just balances the sum of the thermal losses due to radiation, convection, and water vaporization.

Before we can estimate the actual rise of temperature of the leaf under the conditions postulated, we must be in a position to determine the rate of loss of energy due to each one of these causes. We will assume first of all that transpiration is still in abeyance, and that the loss of the evolved energy of respiration is due merely to radiation, air-convection, and air-conduction.

From the results given in another paper* we know that the *thermal emissivity* of the leaf per square centimetre of leaf-surface for "still air" conditions and for a temperature-excess in the leaf over its surroundings of 1° C., is about 0·015 calorie per square centimetre per minute. This "rate of cooling" has, of course, to be doubled for the two sides of the leaf, so that the emissivity of a square centimetre of leaf-lamina under the above conditions amounts to 0·030 calorie per minute per 1° C. excess.

Since the heat of respiration has been shown to be 0·000582 calorie per minute per square centimetre, the temperature which the leaf will attain when it is in thermal equilibrium with its surroundings will be given by dividing this number by the emissivity-factor, *i.e.* $\frac{0 \cdot 000582}{0 \cdot 030} = 0^{\circ}\, 0 \cdot 019$ C.

This is the maximal excess temperature above its surroundings to which the leaf can be raised under "still air" conditions when it is respiring 0·7 c.c. of carbon dioxide per square decimetre per hour, provided transpiration still remains in abeyance; and this temperature-excess will increase *pari passu* with any increase in the respiratory process. Since, however, any rise of temperature in the leaf, no matter how small, will increase the partial pressure of the water-vapour in the interspaces of the leaf, a diffusion potential will be produced, and water-vapour will flow from the leaf into the surrounding air, hence even this small theoretical maximum will never be reached.

---

* Brown and Wilson, *infra*, p. 122.

The effect of *transpiration* on the leaf temperature can be best studied in a general way by supposing the leaf to be under the same conditions as above, but surrounded by air which is *not fully saturated* with aqueous vapour for the temperature θ; and here again, for the purpose of argument, we will assume that at the commencement there is no difference of temperature between the leaf and its suroundings.

These conditions are manifestly *unstable* as a consequence of the excess of the partial pressure of the saturated air of the leaf-interspaces over the partial pressure of the water-vapour in the unsaturated air of the enclosure. Owing to the "diffusion-potential" thus set up water-vapour will diffuse through the stomata if these are in any degree open, and the temperature of the leaf will fall. This will continue until the temperature-gradient between the leaf and its surroundings is steep enough to allow energy to flow into the leaf from without at a rate equal to that of the energy expended in the work of vaporization. A thermal static state will then be re-established which will remain constant as long as the conditions remain unaltered, the leaf assuming a temperature θ_n, which will be *less* than θ, the temperature of the surroundings.

Neglecting for the moment the very slight disturbance due to the exothermic respiratory process, as soon as the above thermal equilibrium has been attained *the amount of water*, Q, *lost by unit-area of the leaf surface in unit-time, is a measure of the energy flowing into the leaf from its surroundings,* and if we know the temperature difference between the leaf and its surroundings, *i.e.*, the temperature gradient $\theta - \theta_n$ we can determine the rate of interchange of energy between the leaf and its surroundings in absolute units for a temperature difference of 1° C., that is to say, the *coefficient of thermal emissivity.*

This method of determining the emissivity to which reference has already been made, is elaborated in an accompanying paper.* When once the constant of emissivity has been determined the value of the temperature difference $\theta - \theta_n$ is calculable, providing we know Q, the amount of water transpired by the leaf for unit-area and unit-time.

* Brown and Wilson, *infra*, p. 122.

As a concrete example of a leaf transpiring in an enclosure under the conditions just postulated, we will assume that the leaf is losing water steadily at the rate of 0·5 gramme per square decimetre per hour, or 0·0000833 gramme per square centimetre per minute. The heat required to vaporize this last-mentioned amount of water at 20° C. is $0·0000833 \times 592·6 = 0·04938$ water-gramme-units (calories), and, excluding the small disturbance of the respiratory process, this must represent, according to the theory of exchanges, the amount of energy entering and leaving a square centimetre of the leaf-lamina in one minute, when the steady thermal state has been attained. If the leaf has a thermal emissivity of 0·015 calorie per square centimetre of leaf *surface* per minute for a 1° C. temperature gradient, the temperature difference, $\theta - \theta_n$, between the leaf-lamina and its surroundings will be $\frac{0·04938}{2 \times 0·015} = 1°·64$ C., when the leaf is transpiring at the rate of 0·5 gramme per decimetre per hour, under "still air" conditions.*

Case B.—*Thermal Relations of a Leaf to its Surroundings when it is Receiving direct Solar Radiation.*

The experimental data which are required for an investigation of the energetics of a leaf receiving direct solar radiation, in addition to those already made use of in Case A (see p. 70), are as follows: (1) The total amount of solar radiant energy incident on a given area of the leaf in a given time; (2) the amount of this radiant energy which is absorbed by the leaf (coefficient of absorption); (3) a measure of the internal work of the leaf due to (*a*) water-vaporization, and (*b*) photosynthesis; and (4) the influence which air currents of definite velocity exercise on the thermal emissivity of the leaf surface.

The total solar radiation falling on the leaf was measured by means of a Callendar's radiometer which was connected with a Callendar's self-recorder. The radiometer was calibrated in water-gramme-units and was placed beside the leaf under experiment with similar orientation. The integration of the thermal curve recorded on the drum was performed by an attached planimeter, the reading of which gave a measure of the average solar radiation falling on unit-area of the leaf in unit-time for the whole period during which the experiment lasted.

The coefficient of absorption of the leaf for solar radiation was determined by means of the same instrument in the manner described in detail later on. It was taken as the difference between the solar radiant energy falling on the leaf in full sunshine and the amount transmitted, and takes no account of any possible *reflection* of radiation from the surface of the leaf. With perpendicular incidence the reflected radiation must be very small in amount, but it is well to bear in mind that, strictly speaking, the value of the coefficient of absorption employed includes this reflected portion. Although the absorptivity varies in the leaves of different plants there is a remarkable constancy in the results obtained at different times with the leaves of plants of the same species.

---

* The influence of movement in the air in increasing the thermal emissivity and in decreasing the temperature difference $\theta - \theta_n$ will be considered later.

The loss of water by the leaves due to transpiration was determined by weighing the leaf and its attached water-tube at suitable intervals.

In those cases where it was desired to submit two leaves to solar radiation of definite relative intensity, the leaf to be partially shaded was placed under the radial arms of a *revolving sector* which could be adjusted for any required degree of "exposure." In this way a leaf could be submitted to a definite fraction of the full solar radiation falling on another leaf placed alongside without the risk of introducing any error from selective absorption of any special rays, which it is difficult to avoid when ordinary screens are used. The area of the leaves was ascertained by the planimeter method described in an earlier part of the paper, and the amount of energy used up in the photosynthetic process was deduced from the rate of assimilation as determined by the absorption of carbon dioxide from the air, a method which has been fully described in Part II.

Before discussing the results of some typical experiments it will be well to consider in a general way the thermal relations of a leaf to its surroundings when it is exposed to sunlight under free air conditions, just as we have considered these relations in the case of a leaf under the still air conditions of an enclosed space cut off from direct solar radiation.

In the first place let us suppose a healthy green leaf, well supplied with water, to be exposed to sunlight under perfectly constant conditions as regards intensity of solar radiation, and the temperature, humidity and degree of movement in the surrounding air; and further that the apertures of the stomata undergo no variation in dimensions. Under these ideal conditions, just as in the case previously considered, a state of thermal equilibrium will be speedily established between the leaf and its surroundings, when the loss and gain of energy by the leaf in a given interval of time will just balance.

Let R represent the total solar radiation expressed in water-gramme-units (calories) falling on a square centimetre of the leaf-lamina in one minute, and let a represent the "absorptive coefficient" of the leaf for this radiation.

Then Ra will represent the radiant energy absorbed per square centimetre of leaf-lamina per minute.

It is worth while at this point to see how the temperature of the leaf would be affected on the assumption that none of this absorbed energy were dissipated. If we denote the mass of a square centimetre of the leaf-lamina by m, and its specific heat by s, the rise of temperature of the leaf-lamina per minute will be represented by Ra/ms.

Taking R, the solar radiation, at the comparatively low value of 0·8 calorie per square centimetre per minute, a, "the coefficient of absorption" of the leaf as 0·78, m the weight of a square centimetre of leaf as 0·020 gramme, and s its specific heat as 0·879; then the rise of the temperature of the leaf under the conditions postulated would be $\frac{0{\cdot}8 \times 0{\cdot}78}{0{\cdot}02 \times 0{\cdot}879} = 35^\circ{\cdot}4$ C. *per minute*, a result which would be speedily fatal to the leaf if there were no means of dissipating the absorbed radiation.

The dissipation of energy necessary to keep within safe limits the temperature of a leaf exposed even to moderate solar radiation is provided for by the internal work performed, and by the loss of heat due to radiation and air-convection. The internal work attended with any sensible absorption of energy is (1) vaporization of water, and (2) photosynthesis of carbohydrates from atmospheric carbon dioxide.

We have a measure of (1) in the water lost by transpiration, and denoting this loss per square centimetre of leaf-lamina per minute by Q, the heat dissipated by vaporization for the same units of leaf-area and time will be **592·6 Q** calories; 592·6 being the latent heat of vaporization of 1 gramme of water in water-gramme-units.*

The energy used up in photosynthesis can be determined from the mass or volume of carbon dioxide absorbed by the leaf under the conditions of the experiment.

The heat of combustion, and therefore with a reversed sign, the heat of formation of the carbohydrate synthesized, must closely approximate 3760 calories per gramme, hence it can readily be shown that the assimilation of 1 cubic centimetre of carbon dioxide, measured at normal temperature and pressure, corresponds to 0·001336 gramme of a hexose, and to the absorption of 0·001336 × 3760 = 5·02 water-gramme-units of energy. If, therefore, we denote the volume in cubic centimetres of carbon dioxide assimilated by

---

* This is the latent heat of vaporization of water at 20° C. The value varies slightly with the temperature, according to the formula $606{\cdot}5 - 0{\cdot}695\, t^\circ$, t° being the temperature in degrees Centigrade.

1 square centimetre of leaf-lamina per minute by c, then the energy used up in photosynthesis, expressed in calories per square centimetre of lamina per minute will be **5·02*c***.

The total internal work due to water vaporization and assimilation together, per square centimetre of leaf-lamina per minute, will therefore be represented in calories by

$$592 \cdot 6\ \mathrm{Q} + 5 \cdot 02c = \mathrm{W} + w,$$

W being the generalized expression for the work effected in water-vaporization, and w that due to the photosynthetic process.

Now $\mathrm{W}+w$, owing to the complex interactions of a variety of conditions, may be equal to $\mathrm{R}a$, the total solar radiation absorbed by the leaf, or it may be less or greater than this value.

If $\mathrm{R}a = \mathrm{W} + w$ it is evident that when the steady thermal condition is attained, the leaf will have the same temperature as its surroundings.

If, on the other hand, $\mathrm{R}a$ exceeds $(\mathrm{W}+w)$, then the excess of solar radiant energy will raise the temperature of the leaf above that of its surroundings, and the steady thermal condition will only be attained when there is a sufficiently high temperature gradient for the excess of energy thus received, to be dissipated by re-radiation and convective cooling. The thermal static condition will in this case be represented by

$$\mathrm{R}a = (\mathrm{W}+w) + r,$$

r, being a measure in calories of the sum of the losses due to radiation and convective cooling, whilst at the same time it is the only portion of R which can produce a rise of temperature in the leaf. It will be observed that whilst $\mathrm{R}a$ and $(\mathrm{W}+w)$ are values obtained by direct experiment, r is a difference value.

The actual rise of temperature of the leaf above its surroundings can be determined from r if we know the *thermal emissivity* of the leaf surface, which is ascertainable. If e is taken to represent this emissivity in air in calories per square centimetre of leaf *surface*[*] per minute, for a difference of 1° between the leaf and its surroundings and, as before, $\theta =$ the temperature of the surroundings, and θn that of the leaf, then, when the thermal static state is attained, the temperature difference $\theta n - \theta = r/2e$.

We have still to consider the case when $\mathrm{R}a$, the absorbed solar radiation, is *less* than $W+w$, representing the sum of the internal work of the leaf. The equation for static thermal equilibrium then becomes

---

[*] Care must be taken to differentiate between the "area of leaf-lamina" and the "surface-area of a leaf." In the above equations R, W, w, and r are in terms of the former, whilst e is in terms of "surface-area" and has, therefore, to be multiplied by two when used for determinations of the temperature of the lamina.

$$Ra+r = W+w.$$

The excess amount of energy requisite for producing the internal work under these conditions *must be drawn from the surroundings of the leaf,* that is to say, the temperature of the leaf must fall below that of its surroundings when thermal equilibrium is reached. The thermometric difference $\theta-\theta_n$ will again be expressed by $r/2e$, as in the preceding case.

The influence of the heat evolved by the respiratory process has still to be considered. The true measure of the photosynthetic work effected by suitable radiation is, strictly speaking, not given by the amount of atmospheric carbon dioxide absorbed by the leaf, but by this amount *plus* the carbon dioxide which would have been evolved by respiration if photosynthesis were in abeyance. But this correction, although one to be taken into account under certain circumstances, does not affect the above thermal equations, since the heat of respiration is opposite in sign to the heat of reformation of the carbohydrate, and these values, representing a concurrent gain and loss of energy by the leaf, must exactly balance each other if the two carbohydrates are identical; and if they are not identical the difference will be so extremely small that it may be safely neglected.

In discussing the thermal relations of a leaf to its surroundings we have so far, for the sake of simplicity, imagined an ideal set of conditions under which all the determining factors, both internal and external, remain constant for a sufficient time to allow of the attainment of static thermal equilibrium. In practice this ideal condition is never attainable. In the first place the incidence of solar radiation, even under the most fair-weather conditions, is subject to rapid oscillations of considerable magnitude, as can readily be seen by watching the constant movement of the pen of the self-recording instrument or by reference to the final "graph" which is integrated by the attached planimeter.

Every variation of this kind alters the value of Ra, the actual amount of energy absorbed by the leaf. Even if these changes are unaccompanied by any alteration in the degree of opening of the stomata, and the external air conditions remain constant, the amount of water vaporization must still be indirectly influenced, since the value of r which represents the difference between the amount of the utilizable incident energy and the internal work performed, must also vary, and as this determines the temperature of the leaf, there will be corresponding changes in the "diffusion-potential" between the inner saturated air of the leaf and the surrounding atmosphere.

Should the variations in solar radiation produce directly or indirectly any effect on the guard-cells which control the stomatic openings, there will be a consequent increase or decrease in transpiration due to this cause, and this will again produce its effect on W and r. The same will also be true of any variation in the hygrometric state of the outer air, and of the slightest change in its temperature. But of even more importance than any of the above mentioned disturbing factors is the influence exerted by variations in the velocity of the air-currents passing over the leaf. These variations act mainly through their effect on the *thermal emissivity* of the leaf, e, and have been the subject of a special investigation* which brings out the important fact that the thermal emissivity of a leaf increases over "still air" conditions by about 0·0017 calorie per square centimetre of leaf surface per minute for every increase in the velocity of 10 metres per minute. Thus a leaf which has an emissivity in still air of 0·015 calorie per square centimetre of leaf surface per minute for a temperature excess of 1° will have this *doubled* by the very moderate air-velocity of 44·2 metres per minute (2·65 kilometres per hour) and trebled at double this velocity. It only requires reference to the formula connecting leaf-temperature with emissivity to see that this would mean a corresponding diminution of the leaf-temperature to one-half and one-third respectively of that in still air, when all other conditions remain the same.

But in addition to the influence of moving air-currents on the "rate of cooling" of the leaf induced by alterations in the thermal emissivity of its two surfaces we have also to consider the direct effect of the currents in promoting transpiration, and here we must draw attention to one very essential point of difference between the loss of water from the surface of an actively transpiring leaf and evaporation from a free surface of water. The difference is one of fundamental importance to the well-being of the leaf and depends on its physical structure. If we imagine a free limited surface of water exposed to evaporation in unsaturated air which is in very slight steady horizontal movement,† the partial pressure of the water-vapour in the air may be regarded as varying in a direction normal to the surface of the liquid from a maximum of ρ_1 at the immediate surface to a partial pressure of ρ at some distance l above the surface, ρ expressing the partial pressure ("tension") of the water-vapour in the surrounding air before it comes under the influence of the evaporating liquid. The gradient of density on which, other things being equal, the rate of evaporation depends, is represented by $(\rho_1-\rho)/l$ so that an increased velocity of the air-current over the liquid surface will *increase the speed of evaporation* by reducing the value of l. This will take place no matter whether the air is moving across the surface of the liquid in a steady stream, or in a turbulent current with vortices, but in the latter case l will assume different values in a plane parallel to the surface of the liquid.

---

* See Brown and Wilson, *infra*, p. 122.

† For the sake of simplicity we have imagined the air to be in very slight movement just sufficient to displace the curved lines of equal density of the over-lying water-vapour and to render these practically horizontal. The exact form which the lines of equidensity would take in perfectly still air over a circular disk of liquid has been fully discussed elsewhere (see Brown and Escombe, 'Phil. Trans.,' B, vol. 193, 1900, p. 223).

There would seem to be no theoretical limit to the increased evaporative power of the air-stream for a given difference of ρ_1 and ρ, until the speed of the air approaches that of the "mean square speed" of the molecules of water leaving the surface, but no doubt other disturbing factors would come in long before this point was reached. It is sufficient for our present purpose to note that, for all ordinary velocities of atmospheric currents, evaporation from a free water surface will be increased by increased speed of the air-current, a deduction which is consonant with known facts.

The loss of water from the surface of a *transpiring leaf* on the other hand does not take place from a free liquid surface, but by stomatal diffusion, that is to say, by diffusion through a series of fine perforations in the lamina which may be regarded as very short tubes connected below with the interspaces of the leaf in which the partial pressure of the water-vapour is at a maximum The conditions of diffusion in a system of this kind, both in still and in moving air, have been fully discussed in a previous paper.*

In *perfectly still air* the rate of diffusion through a tube of area S is expressed by $k\rho\mathrm{S}/(l+2x)$, k being the diffusion constant for water-vapour in air, ρ the density (or partial pressure) of the vapour in the outer air at some point remote from the aperture, l the length of the tube, and x its diameter $\times\frac{1}{8}\pi$. Under the conditions of perfectly still air a series of elliptical "shells" of vapour of equal density is formed over the stomatal aperture, the density increasing from ρ at some point remote from the aperture to ρ_1, at the aperture itself. A very slight current of air is sufficient to disturb this system of "shells" and to produce a density approaching that of ρ at the aperture, when the diffusion of vapour outwards, all other conditions remaining the same, will approximate to $k\rho\mathrm{S}/(l+x)$, *which it cannot exceed no matter how rapid the air-current may be.*

In a leaf stomate of *Helianthus annuus* $l=0{\cdot}0014$ cm., whilst $x=0{\cdot}00042$ cm. *i.e.*, $0{\cdot}3l$, from which it follows that the ratio of diffusivity of water-vapour through the stomata in still and moving air will be $1:1{\cdot}23$ as a maximum, and this maximum will be attained at comparatively low speeds of air current.*

---

* 'Phil. Trans.,' B, vol. 193, 1900, pp. 256 to 260.

But although this statement applies to *stomatal* transpiration, which is responsible for the greater part of the loss of water by the leaf, it is not necessarily true for *cuticular* transpiration which may be more responsive to varying wind velocities. The amount of water brought to the free exterior surface of the cuticle must depend not only on the permeability of the cuticle itself, but also on that of the epidermis and the underlying tissue. The epidermal cells with their large lumina must form a bad water-conducting tissue, especially where they overlie the spongy parenchyma. The recent experiments of Buscalioni and Pollacci† on the absorption of water by collodion-films applied to the surface of the leaf indicate that the portions of the cuticle in apposition to the anticlinal cell-walls of the epidermis absorb more water from below than the other portions, and it seems probable that the supply for cuticular transpiration reaches the surface of the leaf mainly in this way. Notwithstanding the extreme impermeability of the cuticle, the large area which it exposes relatively to the stomatic openings certainly contributes a sensible proportion of the transpired water, and it seems probable that careful observations on the influence of the speed of an air current on the rate of loss of water may enable us to more sharply differentiate the two forms of transpiration which have hitherto been determined only by comparisons of stomatiferous and non-stomatiferous surfaces.

Sufficient has been said above to indicate the complexity of the problem with which we have to deal, and with all the constantly varying factors acting and reacting on each other, it may well be understood that under natural open-air conditions the thermal relations of a leaf to its surroundings must be constantly undergoing re-adjustment, and that the position of the point of "static thermal equilibrium" must change from moment to moment with every passing cloud, with every gust of wind, and with each change in inclination of the plane of the leaf-lamina to the incident radiation.‡

In the absence of means for instantaneously determining all these factors it is manifestly impossible to ascertain the conditions at any particular moment of time, and there would, perhaps, be no great advantage in doing so even if it were possible. It is the *average* values of the varying factors, extending over a given interval of time, say a few hours, which it is more important to know, and from these it is possible to deduce a mean expression for the general equation $Ra = (W + w) + r$, and for the temperature difference $\theta - \theta_n$, or $\theta_n - \theta$.

---

* It is quite possible that, quite apart from the question of diffusion-phenomena, the irregular action of a strong wind on leaves may result in a certain amount of intermittent compression of the leaf-lamina which may produce tidal ebbs and flows of air through the stomata.

† 'Instit. Bot. Univer. Pavia,' vol. 7, 1902.

‡ The intensity of the radiation received on unit-area varies with the sine of the angle of incidence.

We have recorded in the sequel our endeavours to carry out this idea, but the detailed results will be rendered more intelligible by a preliminary discussion of a few typical examples which illustrate the principles enunciated in the present section of the paper.

Simultaneous experiments were made on two similar leaves of *Helianthus annuus* for the purpose of determining (1) the rate of photosynthesis of carbon dioxide in ordinary air; and (2) the amount of water transpired.

The leaves were exposed to intermittent sunshine of somewhat low intensity for about four hours, the total amount of radiation incident upon them being measured by means of the Callendar radiometer placed alongside the leaves.

In (1) the leaf was enclosed in a glazed case through which ordinary air was passed, and the amount of carbon dioxide assimilated was estimated from its determination in the entering and emergent air, the results being corrected to the partial pressure of the carbon dioxide in the outer atmosphere, which in this instance corresponded to 2·71 parts per 10,000 of air.

Leaf (2), for determining the rate of transpiration, was freely exposed to the air, which had an average temperature of 16°·9 C., and an average velocity of 25·7 kilometres per hour. The partial pressure of the water vapour in the air was 9·21 mm. of mercury as determined from the average readings of the wet and dry bulb thermometers. The area of each leaf was, of course, accurately determined.

The results were as follows:—

(R)	Average total solar radiation falling on the insolated leaves per square centimetre per minute	0·2569 calories.
(A)	Coefficient of absorption of the leaves	0·686

The actual amount of solar energy intercepted was therefore

$$Ra = 0·2569 \times 0·686 = 0·1762 \text{ calorie per square centimetre per minute.}$$

The amount of carbon dioxide assimilated, reduced to free air conditions, was 2·134 c.c. per square decimetre per hour, or 0·000355 c.c. per square centimetre per minute, which corresponds to an absorption of $0·000355 \times 5·02 = 0·0017$ calorie per square centimetre per minute (w).

The water transpired from the leaf under free air conditions amounted to 1·259 grammes per square decimetre per hour, or 0·000209 gramme per square centimetre per minute. The amount of internal work of vaporization, W, is therefore $0{\cdot}000209 \times 592{\cdot}6 = 0{\cdot}1243$ calorie per square centimetre per minute.

The total amount of internal work $W + w$ performed by the leaf is therefore equivalent to $0{\cdot}1243 + 0{\cdot}0017 = 0{\cdot}1260$ calorie per square centimetre of leaf-lamina per minute.

In this instance Ra, the solar radiation absorbed by the leaf, exceeds $W + w$, the sum of the internal work of the leaf, by $0{\cdot}1762 - 0{\cdot}1260 = 0{\cdot}0502$, which represents the value of r in calories in the equation

$$Ra = (W + w) + r.$$

This value for r of 0·0502 calorie per square centimetre per minute represents the *only part of the solar radiation which can have had any heating effect on the leaf.* From the value of r we can determine the mean temperature-difference between the exposed leaf and its surroundings $\theta_n - \theta$, if we know the thermal emissivity of the leaf, for $\theta_n - \theta = r/2e$.

The thermal emissivity of a leaf of this nature, for "still air" conditions, is approximately 0·015 calorie per square centimetre of leaf *surface* per minute for a temperature excess of 1°, and the emissivity increases by 0·00017 calorie per square centimetre per minute for an increased air speed of 1 metre per minute. Hence, since the average velocity of the wind in this case was 25·7 kilometres per hour, or 428 metres per minute, the corrected "emissivity" becomes $0{\cdot}0150 + 0{\cdot}00017 \times 428 = 0{\cdot}0577$ calorie per square centimetre of *surface* per minute for a temperature excess of 1°. Hence the temperature excess of the leaf-lamina above its surroundings will be

$$\theta_n - \theta = \frac{r}{2e} = \frac{0{\cdot}0502}{0{\cdot}1154} = 0^\circ{\cdot}43 \text{ C.}$$

Since the average temperature of the air during the experiment was 16°·9 C., that of the leaf was about 17°·3 C.

We are now in a position to state with a fair approach to accuracy the manner in which the leaf has disposed of the energy incident upon it, and to obtain some idea of the "economic coefficient" of the leaf under these average conditions. If we denote R, the total energy incident on unit-area of the leaf in unit-time, by 100, then the disposal of this radiation will be accounted for in the following manner:—

(w).	Energy used for photosynthesis	0·66
(W).	,, transpiration	48·39
$(\mathrm{W}+w)$.	Total energy expended in internal work	49·05
$\mathrm{R}-\mathrm{R}a$.	Solar radiant energy transmitted by leaf ...	31·40
(r).	Energy lost by "thermal emission"...........	19·55
		100·00

The "economic coefficient" of the leaf in the above instance was 49·05 per cent. if we include the *whole* of the internal work done in the leaf. If, on the other hand, as is more convenient, we only take into account the *photosynthetic work* on which the plant relies for the production of new formative material, then the "economic coefficient" was only 0·66 per cent.*

We will now consider a case in which the facilities for the performance of the internal work of vaporization, such as are afforded by fully open stomata, and high temperature and low humidity of the surrounding air, are more than sufficient to utilise the whole of the direct solar radiation absorbed by the leaf. Under these conditions $(\mathrm{W}+w)$ will exceed $\mathrm{R}a$, and the balance of energy required for the internal work will be derived from the environment, the temperature of the leaf falling below that of its surroundings.

The following experiment on leaves of *Helianthus annuus* on a warm dry day in July serves to illustrate the point. The transpiring leaf was placed under the revolving sectors so arranged as to cut off exactly one-half of the total solar radiation which would otherwise have fallen upon it.

R.	Total direct solar radiation falling on the leaf per square centimetre per minute ..	0·2746	calorie.
$\mathrm{R}a$.	Solar radiation absorbed per square centimetre per minute (coefficient of absorption = 0·686)	0·1884	,,
w.	Energy expended in assimilation...........	0·0033	,,
W.	,, ,, transpiration	0·3668	,,
$\mathrm{W}+w$.	Total energy used for internal work	0·3701	,,

Hence the energy (r) which the leaf has derived from its surroundings $= (\mathrm{W}+w)-\mathrm{R}a = 0·1817$ calorie per square centimetre of leaf-lamina per minute.

Notwithstanding the fact that the leaf was receiving an incidence of solar radiation equivalent to 0·2746 calorie per square centimetre per minute, its temperature must in this case have been *lower* than that of its surroundings, and this temperature difference $\theta - \theta_n$ will again be measured by $r/2e$.

---

* In this restricted sense the "economic coefficient" of the leaf is the ratio of the energy utilised by photosynthesis to the total radiation falling on the leaf.

Since the average velocity of the wind during the experiment was 12·0 kilometres per hour (= 200 metres per minute) and the "emissivity" of the leaf under "still air" conditions was 0·015 calorie per square centimetre of surface per minute for a temperature difference of 1°, the "emissivity," e for the above speed of the air is represented by $0{\cdot}015 + 200 \times 0{\cdot}00017 = 0{\cdot}0490$, hence,

$$\theta - \theta_n = 0{\cdot}1817 \div (2 \times 0{\cdot}049) = 1^\circ{\cdot}84 \text{ C.}$$

The mean temperature of the surrounding air being 27°·2 C., that of the leaf was consequently $27{\cdot}2 - 1{\cdot}84 = 25^\circ{\cdot}36$.

If, in this last experiment, we represent the sum of the energy received by the leaf both from solar radiation and from its surroundings by 100, we get the following results:—

(w).	Energy used for photosynthesis	0·72	calorie.
(W).	„ transpiration..............	80·38	„
(W + w).	Total energy expended in internal work...	81·10	„
R − Ra.	Solar radiant energy transmitted by leaf..	18·90	„
		100·0	„

Since the receipt and expenditure of energy must necessarily balance each other for a given period of time it is somewhat instructive to arrange the results in the form of a "Revenue and Expenditure Account" for the leaf. For the last mentioned experiment this comes out as follows:—

	Calories per square centimetre of leaf-lamina per minute.
Revenue account—	
Total solar radiation incident on the leaf	0·2746
Gain of energy from surroundings	0·1817
Total energy reaching 1 sq. cm. of leaf per min. ...	0·4563
Expenditure account—	
Energy used up in photosynthesis	0·0033
„ transpiration	0·3668
Total energy used for internal work.....................	0·3701
Incident solar energy transmitted	0·0862
	0·4563

When a leaf is exposed to full sunshine the radiant energy which is utilised for the photosynthetic process represents only a very small part of the total incident radiation. If we restrict the term "economic coefficient" to the ratio of these two values, the full radiation falling on the leaf being taken as 100, it is evident that the leaf is an extremely wasteful transformer of energy, since it receives a very large amount of superfluous energy which does not contribute to the main function of the leaf, and has to be dissipated by some means.

If for the purpose of argument we assume that photosynthetic work is confined to that portion of the solar spectrum corresponding to the principal absorption-band of chlorophyll, lying between the lines B and C, some idea may be obtained of the maximal theoretical efficiency of a leaf exposed under the most favourable conditions to full sunshine, provided we know the relation of the energy in this restricted portion of the spectrum to the total energy.

The question has already been discussed by one of us from this point of view,* and the conclusion was reached that the maximal "economic coefficient" *for full sunshine* would probably be about 6·5 per cent. if the leaf were in a position to sift out and utilise the whole of the particular grade of energy useful for photosynthetic work. But this implies a set of conditions which can never exist in nature owing to the limits imposed on the assimilatory process by the high state of dilution of the "atmospheric carbon dioxide."

That the photosynthetic rays, even in sunlight of very moderate intensity, are in excess of the power of the leaf to utilise them has been shown by the experiments described in Part II, p. 54, in the first place by the increased assimilatory effect produced under constant or practically constant illumination by increasing the carbon dioxide in the surrounding air, and secondly by observing the rate of photosynthesis in air of fixed carbon dioxide-content when the leaf is submitted to solar radiation of varying and known relative intensity.

It was found, for instance, when solar radiation of an average intensity of about 0·5 calorie per square centimetre per minute was reduced to about one-third of this intensity by passing through a thin canvas screen, forming an artificial "cloud," that it still contained an excess of photosynthetic rays over and above what was necessary to produce maximal assimilation in ordinary air; for by means of the revolving-sector-method the intensity of the radiation could be still further reduced to one-quarter, that is to say, to one-twelfth of the original amount, before there was any sensible diminution in the assimilatory power of a leaf submitted to its influence.†

---

* See 'Pres. Address,' section B, 'Brit. Assoc. Rep.,' 1899, p. 681.

† In passing it may be noted that the ratio which the photosynthetic radiation bears to the total

This excess of photosynthetic radiation in weakened sunlight is a fact of the highest importance to the plant, since it enables the leaf to carry out unimpaired the main function of assimilation in diffuse light, and in sunlight of a considerable degree of obliquity.* It has also an important bearing on the "economic coefficient" of the leaf, for it is manifest that this must vary very much according to the intensity of insolation. The "coefficient" will be at a maximum when the insolation is just sufficient to produce the maximal rate of assimilation for a given partial pressure of carbon dioxide in the air, and will decrease with increased incident radiation. The accuracy of this deduction is shown by the detailed results which follow, especially those obtained by experiments with revolving sectors.

A few words still remain to be said about the dissipation of the superfluous energy absorbed by the leaf. We have already seen that this is brought about by the two processes of water vaporisation (transpiration) and thermal emission. The relative part taken by each of these processes will vary greatly according to the nature of the plant and the surrounding conditions. In the case of plants well adapted to water-conduction and provided with abundant stomata, the transpiratory "safety-valve" no doubt plays the more important part, and the temperature-gradient between the leaf and its environment need never be large in non-saturated air. For instance, in the case of a leaf transpiring at the moderate rate of 500 grammes per square metre per hour, it can readily be shown that the vaporisation of this amount of water will absorb about 0·5 calorie per square centimetre per minute. We have never observed a higher maximal value for solar radiation at Kew than about 1·0 calorie per square centimetre per minute, and for observations extending over several hours it has seldom exceeded 0·5 calorie.

solar radiation ought to be approximately determinable by the use of the revolving radial sectors described in Part II, p. 54.

In the particular case cited above no effect on assimilation was produced until the solar radiation of 0·5 calorie per square centimetre per minute had been reduced by the screening to one-twelfth of its original value, *i.e.*, to 0·041 calorie. The observed rate of assimilation at this point was 2·07 c.c. of carbon dioxide per square decimetre per hour, or 0·00034 c.c. per square centimetre per minute, which must mark the stage at which practically the whole of the rays capable of producing photosynthesis were utilised for that purpose. From what has been said before it follows that this amount of assimilated CO_2 corresponds to an absorption of energy of $0{\cdot}00034 \times 5{\cdot}02 = 0{\cdot}0017$ calorie per square centimetre per minute. Since no selective absorption has taken place in the screening process, the proportion of photosynthetic rays in the reduced radiation falling on the leaf, *i.e.*, $\frac{0{\cdot}0017 \times 100}{0{\cdot}041} = 4{\cdot}1$ per cent., also represents the percentage of photosynthesizing energy in the original unscreened solar radiation.

* It follows that the limiting factor in assimilation in ordinary sunlight of even a low degree of intensity is the high degree of dilution of the atmospheric carbon dioxide.

Where transpiration is at a minimum, especially in the extreme case of xerophytic plants, the dissipation of the absorbed radiant energy by *thermal emission* becomes all important, and we are now able for the first time to apply certain numerical values to the dissipation of energy from this cause, which are of some interest.

Since the emissivity of a leaf surface approximates to 0·015 calorie per square centimetre of surface for a temperature excess of 1°, a rise in temperature of the leaf of only 10° will, even under *still air* conditions, give a dissipation of energy equivalent to $10 \times 2 \times 0{\cdot}015 = 0{\cdot}3$ calorie per square centimetre per minute, and in a gentle breeze of only 10 kilometres per hour, this emission will be increased to 0·864 calorie per square centimetre per minute. Hence in the thermal emissivity of a plant we have a vary potent factor for keeping down the temperature, even in the absence of transpiration.

Hitherto we have been regarding only those cases in which the incidence of solar radiation is *normal* to the leaf surface, conditions which seldom occur in nature, especially when radiation is powerful, as in the tropics or at high altitudes, where the self-adjusting mechanism of the plant, and its habit, are such as seldom to allow the leaf to be placed in a position favourable for receiving the full solar radiation.*

Since the intensity of the radiation received on unit-area *varies with the sine of the angle of incidence,* a radiation equal to 1 calorie per square centimetre per minute will be reduced to 0·707 calorie at an obliquity of 45°, and to 0·5 calorie, or one-half of its normal intensity, at an obliquity of 30°.†

Section (2).—*Experimental.*

(a) *Determination of R, the total incident Radiation.*

The total solar radiant energy incident on a leaf in a given time is very conveniently measured by the Callendar's radiometer and recorder already referred to in Part I (see p. 39). This instrument is particularly well adapted to experiments of this kind, since it receives and records the sky-radiation as well as that of direct sunlight, and the area covered by the receiving coils of the differential thermometer is comparable in magnitude with the areas of the leaves employed. Instruments of the type of the Ångström's compensating pyrheliometer, in which the radiation is received through narrow slits or diaphragms, to the almost complete exclusion of sky-radiation, are not so suitable for investigation of this nature.

---

* On this point *cf.* A. J. Ewart on the "Effects of Tropical Insolation" ('Annals of Bot.,' vol. 11, p. 439), who states from observation that "no tropical plant places or allows its leaves to be in such a position that the upper surfaces are at right angles to the sun's incident rays when at the zenith."

† With increasing obliquity there will also be less penetration of the radiations owing to increased reflection. In the case of light, whilst the reflection from the surface of glass is about 2·5 per cent. for normal incidence, it is 3·4 per cent. for an obliquity of 50°, and as much as 29·9 per cent. at an obliquity of 15°.

The radiometer was always exposed in close juxtaposition to the leaf, and in the same plane as the leaf-lamina, so as to ensure equality in the amount of energy received by the two surfaces.

At the conclusion of an experiment, the integrated record, as given by the planimeter reading, was reduced to *water-gramme-units of energy* (*calories*) *incident on one square centimetre per minute.*

As examples of some of the highest readings which we have obtained in this way in full sunshine, we may give the following:—

Table I.

Date.	Time.	Solar radiation in calories per square centimetre per minute.
July 10, 1900......	4.0 P.M.	0·972
„ 17, „	12.20 „	1·019
June 27, 1901......	12.10 „	0·934
„ 27, „	2.20 „	0·941
July 20, „	12.20 „	0·932

The observations above were made on exceptionally clear days, and with the sun's rays normal to the receiving instrument. The values are somewhat

Table II.

Date.	Duration of observation in hours.	Percentage of sunshine.	Mean solar radiation in calories per square centimetre per minute.
June 25, 1901.........	4·30	46	0·246
July 9, „	2·54	100	0·479
„ 11, „	1·93	100	0·549
„ 15, „	0·90	70	0·393
„ 17, „	2·59	76	0·499
„ 18, „	1·55	100	0·550
„ 19, „	1·26	75	0·533

low, a result which is no doubt due to the low-lying position of Kew Gardens and to the absorptive influence of the attenuated veil of London smoke in the lower regions of the air.

When observations are extended over several hours, the mean result, even on the clearest day, seldom exceeded about 0·550 calorie per square centimetre per minute, as is shown by Table II.

The column headed "percentage of sunshine" gives the proportion of the total time occupied by the experiment, during which the sunshine was of sufficient intensity to produce a record on the Campbell-Stokes' burning recorder of the Kew Observatory.

(b) *The Absorption of Solar Radiation by the Leaf-lamina: Determination of* a, *the "coefficient of absorption" of the leaf.*

As a necessary preliminary to the discussion of the quantitative relations between the energy incident on the leaf and the internal work produced, we must determine the respective proportions of the radiant energy of sunlight which are transmitted and absorbed by the leaf-lamina.

This *coefficient of absorption*, a value which we have denoted by a in the general thermal equations of the leaf referred to in Section (1), was determined in the following manner:—

Over the glass cover of the Callendar's radiometer was fixed a closely-fitting cardboard-cover, out of which a square opening was cut of a sufficient size to expose the platinum-spirals, the opening being bisected by the division between the spirals. The leaf under experiment, with its petiole dipping into a small tube of water, was then laid down on the cover of the radiometer in such a manner that the mid-rib* lay between the spirals and the leaf-lamina was symmetrically disposed over the coils. Over the leaf there was then placed another piece of cardboard with a similar square opening, and when this was tied down to the radiometer the lamina was in the right position.

A favourable day of bright sunshine was selected for the experiment, when we could rely on the solar radiation remaining constant, or nearly so, during the few minutes required for an observation.

The plan adopted was in the first place to obtain a measure of the unobstructed solar radiation falling normally on the platinum-spirals, waiting until the pen of the recorder drew a straight line on the drum. Another similar observation was then made after the interposition of the leaf-lamina, and finally a second observation of the unobstructed radiation. The effect produced by the leaf was referred to the mean of the first and third observations with full radiation, and the result was expressed in the form of a *coefficient of absorption* (a) for the leaf-lamina, the full radiation falling on the leaf being taken as unity.

---

* To facilitate the leaf being brought into close apposition to the glass cover of the radiometer, slits were cut in opposite sides of the cardboard frame in order to receive the mid-rib.

The only sensible error to which this method is liable is that due to *reflection* of radiation from the leaf-surface, an error which would tend to unduly increase the estimated coefficient of absorption. With the perpendicular incidence of sunlight such as was generally employed in these experiments the amount of reflection must, however, have been very small.

The estimation of the coefficient of absorption cannot be perceptibly influenced by re-radiation from the leaf to the platinum-spirals, since, in the first place, during the very short time the experiment lasts, the rise of temperature of the lamina will be very small, especially as transpiration is going on; and, secondly, the glass cover of the radiometer is very opaque to the obscure radiation which the leaf would emit.

With the same leaf and at different times of the day the method gives very concordant results, as will be seen from the following obtained with a leaf of *Helianthus annuus.* In these experiments the actual values of the unobstructed solar radiation (R) varied from 0·591 to 0·636 calorie per square centimetre per minute.

	Coefficient of absorption for sunlight.
Helianthus annuus (1)..................	0·687
„ „ (2)..................	0·689
„ „ (3)..................	0·684

Although the coefficient of absorption is fairly constant for mature and healthy leaves of the same species of plant it shows considerable variation in the leaves of different species, as will be seen from the following Table III, in which are given the mean values for a number of different plants. The values of the *coefficients of transmission* (*i.e.*, $1-a$) are also given in this instance.

A series of observations was now undertaken in order determine how far individual leaves of the same plant differ in their coefficient of absorption for solar energy, and in order to bring out any possible correlation which may exist between this property and the age of the leaf, the experiments were carried out on leaves taken from the plant in the *serial order of their development.*

Table III.—Coefficients of Absorption and Transmission of the Radiant Energy of Sunlight for Leaves of Different Plants.

Plant.	Coefficient of absorption (a).	Coefficient of transmission ($1 - a$).
Helianthus annuus	0·686	0·314
Polygonum Weyrichii	0·647	0·353
,, *Sachalinense*	0·691	0·309
Petasites officinalis	0·728	0·272
Silphium terebrinthaceum	0·699	0·391
Arctium majus	0·728	0·272
Verbascum olympicum	0·758	0·242
Senecio grandifolius	0·774	0·226

The following table gives the results of two different series of observations of this kind made on leaves of *Senecio grandifolius* :—

Table IV.—Coefficient of Absorption of the Radiant Energy of Sunlight for Leaves of *Senecio grandifolius* taken from the same Plant in Serial Order.

	Series A. Coefficient of absorption.	Series B. Coefficient of absorption.
Leaf 1 (youngest mature leaf)	0·750	0·776
,, 2	0·748	0·771
,, 3	0·757	0·763
,, 4	0·762	0·784
,, 5	0·793	0·776
,, 6	0·778	—

It will be seen that the variations in the values of the coefficient of absorption in this plant are not large, and that such as they are they show little or no relation to the age of the leaf.

In another similar series of experiments with *Polygonum Sachalinense*, recorded in the following table, there was some indication of a slight increase of the coefficient of absorption with the age of the leaf.

Table V.—Coefficient of Absorption of the Radiant Energy of Sunlight for Leaves of *Polygonum Sachalinense* taken from the Plant in Serial Order.

	Coefficient of absorption.
Leaf 1 (youngest)	0·687
„ 2	0·681
„ 3	0·696
„ 4	0·702

(c) *The Selective Absorption of Radiant Energy by the Leaf.*

In considering the general thermostatic and thermodynamic problems arising out of the reception and utilization of radiant energy by the leaf, we have so far only been concerned with the gross amount of energy received, irrespective of its particular grade. As long as we confine ourselves to the consideration of *single* leaves the principles which have hitherto guided us are sufficient to determine the thermal relations of a leaf to its environment without reference to the particular quality of the rays transmitted or absorbed by the leaf-lamina. On the other hand, any attempt to determine the thermal and other effects induced by sunlight which has already been filtered through one or more leaves must necessarily take into account the property of *selective absorption* possessed by the lamina, a property which is mainly due to the colouring matter of the leaf.

If the leaf-lamina absorbed equal proportions of the various undulations incident upon it in the form of solar radiation, that is to say, if it possessed no power of selective absorption, we should expect the transmitted portion of the radiation to diminish in geometric proportion as the number of similar leaves was increased in arithmetic proportion. Thus, taking the incident radiation as unity, and assuming that we have a leaf with a coefficient of absorption of 0·687, and therefore a coefficient of transmission of 0·313, if selective absorption does not come into play we ought to obtain a transmission through *two* superimposed and similar leaves of $0{\cdot}313^2 = 0{\cdot}098$, with *three* such leaves of $0{\cdot}313^3 = 0{\cdot}030$, and with n leaves a transmission of $0{\cdot}313^n$.

In a green leaf, however, we have to deal with a screen which is eminently selective in its absorbing power, a fact which is clearly brought out by the results given in the following table, which show the increased transmission by a second and third leaf of the heat rays which have passed through the first. The experiments were made with the Callendar's radiometer by the successive superposition of the leaves.

Table VI.—Proportion of the Radiant Energy of Bright Sunshine Absorbed and Transmitted by the Successive Superposition of Three Leaves.

Full unscreened radiation = 100.

Name of plant.	Radiation absorbed. Per cent. of total.	Radiation transmitted. Per cent. of total.	Radiation which would have been transmitted in absence of selective absorption.
Series A. *Polygonum Weyrichii*, coefficient of absorption, 0·647—			
One leaf	64·7	35·3	—
Two leaves	80·8	19·2	12·4
Three leaves	84·5	15·5	4·4
Series B. *Helianthus annuus*, coefficient of absorption, 0·687—			
One leaf	68·7	31·3	—
Two leaves	82·6	17·4	9·7
Three leaves	88·4	11·6	3·0

The selective absorption of solar radiation by the green colouring matter of leaves, also comes out very clearly in certain experiments described by Timiriazeff, and referred to further on. He found, for example, that whilst the chlorophyll which had been dissolved out of a given area of a Maple leaf arrested 27 per cent. of the energy of direct sunlight, this absorption was only increased to 31 per cent. by a triple concentration of the solution.

(d) *Relative absorption of Solar Radiation by Albino and Green leaves of the same plant.*

The plant selected for these experiments was *Negundo aceroides*, and since the leaves were too small to be used with the Callendar's radiometer in the manner already described, other means had to be devised for determining the coefficient of absorption of the leaf. The experiments were kindly undertaken by Dr. W. E. Wilson, and were carried out during the past summer at his Observatory at Daramona, Westmeath.

In the first instance an attempt was made to employ the Boys' radiomicrometer, by reflecting a portion of the sun's image from a heliostat on to the suspended thermo-electric junction, and taking successive readings of the galvanometer with and without the interposition of the leaf-lamina. It was found, however, that under these conditions both the white and the green leaves acted as perfect screens, and completely prevented any sensible amount of solar radiation from reaching the sensitive part of the instrument.

The same difficulty was also experienced in attempting to adapt the Angström's pyrheliometer to the purpose, the failure in both cases being due to the fact that the absorbing leaf-lamina, which must necessarily be at some little distance from the sensitive thermo-electric junction, so far scatters the radiation which passes through the small apertures of the instruments, that the vertical component of the transmitted rays, which can alone affect the instrument, becomes very small. The aperture covered by the leaf becomes, in fact, a new focus of radiation which spreads the rays over an area much larger than that occupied by the receiving surface of the sensitive portion of the instrument.

It is necessary, therefore, that the receiving surface forming the thermometer should have, as in the Callendar's instrument, a considerable extension, and should be well overlapped by the leaf, which must admit of being brought close to the receiving surface.

For leaves too small for the Callendar's radiometer, a Rubens' thermopile was found to be suitable, the leaf being placed between two plates of thin glass and brought within about a millimetre of the receiving end of the pile. The absorptivity of the leaf for bright sunshine was determined by observing the amount of radiation transmitted through the glass plates, both with and without the interposition of the leaf.

A series of concordant experiments made in this manner with the white and green portions of a leaf of *Negundo*, gave the following mean results in bright sunshine:—

	Transmission.
Through glass alone	100·0
White leaf interposed	25·5
Green „ „	21·3

These results lead to the following values for the coefficients of absorption and transmission for the white and green portions of the leaf respectively.

Table VII.—Absorption and Transmission of the Radiant Energy of Sunlight by the White and Green portions of the Leaf of *Negundo aceroides*.

	Coefficient of absorption.	Coefficient of transmission.
White leaf lamina	0·745	0·255
Green „ „	0·787	0·213

If the transmission through the albino-leaf is taken as 100, that of the green leaf will be represented by 83·5, a difference of 16·5 per cent., which may be regarded as expressing the increase in the absorptive power due to the colouring matters of the leaf.

In connection with the increased absorption induced by the leaf-chlorophyll, it is of interest to compare the above results with those of Timiriazeff, which were obtained by a totally different method.*

Timiriazeff's mode of procedure was to punch out of a leaf a circular piece of known area, and to dissolve its green colouring matter in a volume of alcohol just sufficient to fill a glass cell of exactly the same cross-section as the area of the leaf. The absorptive power of the coloured solution for the radiant energy of sunshine was then determined by means of a delicate thermopile, the absorption for the glass and solvent being ascertained separately. The difference between the two readings was taken as representing the absorption due to the chlorophyll.

Timiriazeff obtained by this method the following results for the absorption induced by the chlorophyll of single leaves.

	Absorption of direct sunlight.
Maple	27·0 per cent.
Lime	29·0 ,,
Oak	23·5 ,,
Plantain	23·4 ,,
Potamogeton	20·0 ,,

The leaf of the *Negundo* was not included in Timiriazeff's experiments, but a consideration of his results points to the probability of this leaf showing by his method a chlorophyll-absorption of more than the 16·5 per cent., which was the amount we found by direct observations on the leaf-lamina.

The widely different methods of experiment would account for this, since the chlorophyll when in solution is under more favourable conditions for exerting its maximum absorptive power than it is under the natural conditions which exist in the living leaf. In the latter case, owing to the particulate nature and scattered distribution of the chlorophyll-bodies, the transmitted radiation must be subjected to a less complete selective absorption than when the chlorophyll is in a state of solution.

---

* See Croonian Lecture, 'Roy. Soc. Proc.,' 1903, p. 449.

(e) *The Determination of the Thermal Emissivity (e) of the Leaf-lamina.*

For a full consideration of the experimental methods adopted for a determination of the thermal emissivity of a leaf-lamina reference must be made to a separate communication on the subject.*

The mean value of the emissivity for the four kinds of leaf examined was for, "still air" conditions, 0·0145 calorie per square centimetre of leaf surface per minute for a temperature excess of 1° C. In moving air the emissivity increases by 0·000172 calorie per square centimetre per minute for every metre per minute increase in the velocity of the air moving over the surface of the leaf-lamina.

(f) *Details of Experiments on Leaves submitted to Solar Radiation of known Intensity, showing the mode of Disposal of the Incident Energy under Defined Conditions.*

The data of these experiments have been brought together in Tables VIII, IX, X, and XI. Their general arrangement will be understood from what has been said in Section (1) Part III on the thermal relations of a leaf to its surroundings, but some detailed explanation of the headings of the columns is desirable at this point.

Tables VIII and IX contain the results of a number of experiments on leaves of different species of plants submitted to solar radiation of known intensity, whilst a direct determination was made of the internal work of photosynthesis and of transpiration.

In order that the assimilatory experiments should be carried on under favourable conditions of temperature, the full solar radiation was, in most cases, modified by passing it through a thin canvas screen before it reached the leaf.

In Table VIII Columns (1) and (2) require no comment.

Column (3) headed "percentage of sunshine," indicates the proportion of the time occupied by the experiment, during which the unobstructed solar radiation was sufficiently intense to be recorded on the Campbell-Stokes burning recorder.

Column (6) gives the average partial pressure of the water-vapour in the air as deduced from the reading of the wet and dry bulb thermometers as given in Columns (4) and (5).

Column (7) gives the average humidity of the air when that of saturated air = 100.

---

* See Brown and Wilson, *infra*, p. 122.

Column (8) gives the average velocity of the wind during the experiment in kilometres per hour.

Column (9) headed "difference of temperature between leaf and air" requires some explanation. The values are deduced, in the first place, from the difference between Ra, the energy absorbed by the leaf, and $W+w$, the energy used up in the internal work; and, secondly, from the known *thermal emissivity* of the leaf in an air-current of the mean velocity of the wind during the experiment. If the emissivity of the leaf per square centimetre per minute for a temperature-excess of 1° be represented by e, then the temperature difference between the leaf and the surrounding air will be represented by $\frac{Ra-(W+w)}{2e}$. If Ra exceeds $W+w$ the temperature of the leaf will be *above* that of its surroundings, whereas if Ra is less than $W+w$ the leaf temperature will be the lower.

The values of Column (10), headed "estimated temperature of the leaf," were obtained from the dry bulb temperatures by adding or subtracting the values of Column (9).

Column (11) gives the excess of the partial pressure of the water-vapour in the air-spaces of the experimental leaf over that of the outside atmosphere. It is, in fact, the differential partial pressure, measured in millimetres of mercury, between the point of saturation corresponding to the leaf temperature as given in Column (10) and the partial pressure of the water-vapour of the outer air as given in Column (6). It is a measure of the "diffusion-gradient" of the water-vapour existing between the interspaces of the leaf and the external air. Assuming all other conditions to be identical the rate of transpiration should be proportional to the values given in this column.

Column (12) gives the rate of photosynthesis determined directly in the manner already fully described. The results are given in terms of cubic centimetres of atmospheric carbon dioxide utilised by one square decimetre of the leaf in one hour.

Column (13) gives the rate of transpiration as directly determined, the results being expressed in grammes of water transpired by one square decimetre of leaf-lamina in one hour.

Columns (14) to (21) include the results obtained by means of the Callendar's radiometer and give an account of the manner in which the incident radiation has been utilized by the leaf. The values are here given in terms of water-gramme-units (calories) per square centimetre of leaf-lamina per minute.

Column (14) gives a measure of the total solar radiation incident on the leaf, a value which has been denoted by R in the general thermal equations.

Column (15) gives the coefficient of absorption of the leaf for solar radiation, this coefficient being denoted by a.

Column (16) gives the proportion of incident solar radiation which is absorbed by the leaf, and which must manifestly be equivalent to the incident radiation multiplied by the coefficient of absorption, *i.e.*, to Ra.

The amount of energy *transmitted* through the leaf will, of course, be represented by $R-Ra$.

Column (17) gives the amount of energy used up by the endothermic process of photosynthesis.

As already explained in Section (1) Part III (p. 77), these values are deducible from the volume or mass of carbon dioxide assimilated by the leaf as given in Column (12). The volume of carbon dioxide assimilated per square centimetre of leaf-lamina per minute, when multiplied by 5·02, gives a measure of the energy w used up in photosynthesis, expressed in calories for the same unit-area and unit-time.

Column (18) in the same manner gives a measure of the amount of energy, W, expended in the vaporization of water by the leaf. The values are deduced from the experimental results of Column (13), giving the water transpired from a given area of leaf in a given time.

The numbers are obtained by multiplying the grammes of water transpired by one square centimetre of the leaf-lamina per minute by the value for the latent heat of water, which at 20° is 592·6 calories.

Column (19) gives a measure of the expenditure of energy for the total internal work of the leaf, both for protosynthesis and water vaporization—*i.e.*, $W+w$. The values given are the sum of those of Columns (17) and (18).

Column (20) gives the actual amount of energy lost per unit-area of leaf-lamina and unit-time owing to re-radiation and the conductive and convective properties of the surrounding air. This is the difference between Ra and $W+w$, when Ra is the greater. It is the only portion of the incident radiation which can produce any rise of temperature in the leaf.

When Ra, the incident radiation falling on the leaf and absorbed by it, is less than $W+w$, the energy used up in internal work, it is manifest that the leaf must draw upon its surroundings for the balance. Where this condition of things exists it is recorded in Column (21). The leaf is then *lower* in temperature than its surroundings.

The results tabulated in Columns (14) to (21) of Table VIII, giving the actual loss and gain of energy by the insolated leaf per square centimetre per

Table VIII.—First Series of Experiments on Leaves under Varying the Leaves and

No. of experiment and date. (1)	Species of plant and general conditions of experiment. (2)	Meteorological data. Percentage of sunshine recorded. (3)	Dry bulb thermometer. (4)	Wet bulb thermometer. (5)	Partial pressure of water-vapour in air in millimetres of mercury. (6)	Degree of humidity of air. Saturated air = 100. (7)	Velocity of wind in kilometres per hour. (8)	Difference of temperature between leaf and air, °C. (9)
			°	°				°
I. June 29, 1900	*Polygonum Weyrichii*— Intermittent sunlight without any screen. Duration of experiment, 4·75 hours	72	20·5	16·5	9·09	69	24·4	+1·98
II. June 19, 1900	*Polygonum Weyrichii*— Leaves exposed to full sunshine under thin canvas screen. Duration of experiment, 5 hours	80	21·1	14·8	8·87	47	26·5	+0·10
III. June 22, 1900	*Polygonum Weyrichii*— Intermittent sunlight. Leaves under thin canvas screen. Duration of experiment, 4·2 hours	56	18·3	12·7	8·13	51	25·7	+0·05
IV. July 3, 1900	*Polygonum Weyrichii*— Intermittent sunshine with some showers. Leaves under canvas screen. Duration of experiment, 4·3 hours	42	16·8	13·8	9·98	70	10·6	+0·44
V. July 11, 1900	*Polygonum Weyrichii*— A hot cloudless day. Leaves exposed under canvas screen. Duration of experiment, 4·9 hours	100	27·2	19·6	12·33	44	26·5	+0·15
VI. Sept. 4, 1900	*Tropæolum majus*— Diffused light with very little sunshine. Leaves exposed under canvas screen. Duration of experiment, 3·3 hours	3·3	17·0	12·7	8·58	60	9·1	+0·58
VII. Sept. 7, 1900	*Tropæolum majus*— Leaves in sunlight under canvas screen. Duration of experiment, 5 hours	55	20·1	14·7	9·49	53	5·4	+1·27

Conditions of Insolation, Illustrating the Thermal Interchanges between their Surroundings.

Estimated temperature of leaf. (10)	Excess of partial pressure of water-vapour in leaf over that of air in millimetres of mercury. (11)	*Assimilation* in cubic centimetres of CO_2 per square decimetre per hour. (12)	*Transpiration* in grammes of water per square decimetre per hour. (13)	Loss or gain of energy by the leaf in calories per square centimetre of leaf-lamina per minute.							
				R. Solar radiation incident on leaf. (14)	*a*. Coefficient of absorption. (15)	R*a*. Solar radiation absorbed by leaf. (16)	*w*. Energy expended in assimilation. (17)	W. Energy expended in transpiration. (18)	W + *w*. Total energy used for internal work. (19)	*r*. Energy lost by re-radiation and air-convection. (20)	Energy received from surroundings. (21)
° 22·4	11·02	3·20	0·599	0·6120	0·647	0·3959	0·0026	0·0592	0·0618	0·3341	—
21·2	9·82	3·758	1·054	0·1942	0·647	0·1256	0·0031	0·1041	0·1072	0·0184	—
18·3	7·49	3·058	0·868	0·1503	0·647	0·0972	0·0025	0·0857	0·0882	0·0090	—
17·2	4·59	2·271	0·517	0·1431	0·647	0·0926	0·0019	0·0510	0·0529	0·0397	—
27·3	14·61	1·479	1·291	0·2418	0·647	0·1565	0·0012	0·1275	0·1287	0·0278	—
17·5	6·27	1·498	0·1410	0·0889	0·700	0·0622	0·0012	0·0139	0·0151	0·0471	—
21·3	9·31	2·078	0·2430	0·1461	0·700	0·1022	0·0017	0·0240	0·0257	0·0765	—

Table VIII—

No. of experiment and date.	Species of plant and general conditions of experiment.	Meteorological data.						Difference of temperature between leaf and air, °C.
		Percentage of sunshine recorded.	Dry bulb thermometer.	Wet bulb thermometer.	Partial pressure of water-vapour in air in millimetres of mercury.	Degree of humidity of air. Saturated air = 100.	Velocity of wind in kilometres per hour.	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
			°	°				°
VIII. Sept. 11, 1900	*Tropæolum majus*— Leaves in sunlight under canvas screen. Duration of experiment, 4·8 hours	95	17·6	12·2	7·78	55	21·8	+0·49
IX. June 6, 1900	*Petasites albus*— Leaves in sunlight under canvas screen. Duration of experiment, 3·2 hours	97	21·1	14·4	8·74	46	12·2	+0·96
X. June 26, 1900	*Petasites albus*— Leaves in weak diffused sunlight under canvas screen. Duration of experiment, 5·3 hours	16	15·5	11·9	8·44	64	25·4	+0·07
XI. July 5, 1900	*Petasites albus*— Leaf in full sunlight for 4/10 of time; afterwards under canvas screen. Duration of experiment, 5·2 hours	45	19·5	16·6	12·11	71	16·0	+0·54
XII. Aug. 7, 1900	*Helianthus annuus*— Leaf still attached to plant for assimilation experiment. No screen used. Duration of experiment, 5 hours	50	16·9	13·2	9·21	65	25·7	+0·28
XIII. Aug. 11, 1900	*Helianthus annuus*— Full solar radiation without any screen. Detached leaves. Duration of experiment, 3·7 hours	87	19·4	14·2	9·24	54	7·4	+1·54

N.B.—In the case of experiments marked thus * the *continued.*

Estimated temperature of leaf. (10)	Excess of partial pressure of water-vapour in leaf over that of air in millimetres of mercury. (11)	*Assimilation* in cubic centimetres of CO_2 per square decimetre per hour. (12)	*Transpiration* in grammes of water per square decimetre per hour. (13)	Loss or gain of energy by the leaf in calories per square centimetres of leaf-lamina per minute.							
				R. Solar radiation incident on leaf. (14)	*a*. Coefficient of absorption. (15)	*Ra*. Solar radiation absorbed by leaf. (16)	*w*. Energy expended in assimilation. (17)	W. Energy expended in transpiration. (18)	W + *w*. Total energy used for internal work. (19)	*r*. Energy lost by re-radiation and air-convection. (20)	Energy received from surroundings. (21)
° 18·1	7·64	1·210	0·1340	0·1282	0·700	0·0897	0·0010	0·0132	0·0142	0·0755	—
22·0	10·89	2·565	0·3373	0·1802	0·728	0·1311	0·0021	0·0333	0·0354	0·0957	—
15·6	4·73	1·879	0·7119	0·1171	0·728	0·0852	0·0015	0·0703	0·0718	0·0134	—
20·0	5·25	2·507	0·6614	0·1834	0·728	0·1335	0·0021	0·0653	0·0674	0·0661	—
17·1	5·27	2·134	1·259	0·2569	0·686	0·1762	0·0017	0·1243	0·1260	0·0502	—
20·9	9·11	*2·40	3·962	0·7341	0·686	0·5035	0·0020	0·3913	0·3933	0·1102	—

values were estimated, not obtained by direct experiment.

minute, are rearranged in a more convenient form in Table IX, where the total amount of energy received by the leaf-lamina is represented by 100, and is accounted for by the two modes of internal work in the leaf, the energy lost by transmission through the lamina, and that lost by re-radiation and the cooling action of the surrounding air.

The percentage of energy used in photosynthesis, w, may be regarded as the true "economic coefficient" of the leaf—that is to say, the percentage of the total incident energy which, under the particular conditions of each experiment, the mechanism of the leaf-lamina has been able to transform into useful photosynthetic work contributing to the production of new material for the plant.

The experiments recorded in Tables VIII and IX were carried out on different days on which the insolation and atmospheric conditions varied, so that they are not comparable with each other except in a general way, even where leaves of the same species of plant were employed.

In the second series of experiments, the details of which are given in Tables X and XI, simultaneous duplicate experiments were made on similar leaves for which all the external conditions were the same except the degree of insolation represented by R. Whilst one leaf, A, was exposed to the full sunshine, the other, B, was exposed behind revolving sectors which cut off a definite proportion of the solar radiation. Thus, as far as regards the conditions which were under control, there was only one variable introduced, that of R, and the two members of each pair of experiments are therefore capable of being compared with each other from this point of view. It must not, however, be lost sight of that variations in the value of R tend to introduce secondary effects on the internal work of transpiration, not only by altering the temperature of the leaf, but also indirectly by altering the degree of opening of the stomata. Although, therefore, such experiments as those recorded in Tables X and XI admit, under the controlled conditions of incident radiation, of a much closer comparison than those of Tables VIII and IX, still it is practically impossible to produce conditions under which only one factor at a time shall vary, owing to the mutual dependence of the various factors one on the other, a fact which has already been fully discussed in Section (1), Part III.

The experiments of Series A of Tables X and XI were all carried out under the full available insolation, but, notwithstanding this fact, it will be noted that the condition of the stomata and the excess of partial pressure of water-vapour between the interspaces of the leaf and the surrounding air were so favourable that the energy required for the internal work of transpiration so

Table IX.—The Summarized Results of Table VIII, showing the Mode of disposal by the Leaf of the Energy flowing into it.

The Total Energy received = 100.

	Expt. I.	Expt. II.	Expt. III.	Expt. IV.	Expt. V.	Expt. VI.	Expt. VII.
w. Energy used for photosynthesis	0·42	1·59	1·66	1·32	0·49	1·34	1·16
W. ,, transpiration...	9·67	53·60	57·01	35·64	52·72	15·64	16·42
W + *w.* Total energy expended in internal work	10·09	55·19	58·67	36·96	53·21	16·98	17·58
R − R*a.* Solar radiant energy lost by transmission through leaf	35·31	35·30	35·32	35·28	35·30	30·00	30·00
r. Energy lost by re-radiation and air-convection	54·60	9·51	6·01	27·76	11·49	53·02	52·42
	100·00	100·00	100·00	100·00	100·00	100·00	100·00

	Expt. VIII.	Expt. IX.	Expt. X.	Expt. XI.	Expt. XII.	Expt. XIII.
w. Energy used for photosynthesis	0·78	1·16	1·28	1·14	0·66	0·27
W. ,, transpiration...	10·21	18·47	60·03	35·60	48·39	53·30
W + *w.* Total energy expended in internal work	10·99	19·63	61·31	36·74	49·05	53·57
R − R*a.* Solar radiant energy lost by transmission through leaf	30·03	27·20	27·24	27·20	31·40	31·40
r. Energy lost by re-radiation and air-convection	58·98	53·17	11·45	36·06	19·55	15·03
	100·00	100·00	100·00	100·00	100·00	100·00

far exceeded the reception and absorption of solar energy, that the temperature of the leaf was lower than the surrounding air; in other words, the leaf was receiving energy from its surroundings in addition to that of the solar radiation incident upon it.

Owing to the desirability in these last-mentioned experiments of employing solar radiation of considerable intensity, direct control-experiments on assimilation could not be carried out simultaneously as they were in the previous experiments of Tables VIII and IX, where the insolation had been purposely lowered in intensity by suitable screening.

A close estimate of the rate of assimilation could, however, be arrived at from previous experiments, and the error introduced into the thermal results by this cause must be very small.

Table X.—Second Series of Experiments on the Thermal Interchanges between by *Revolving Sectors* Arranged to Cut Off a

No. of experiment and date. (1)	Species of plant and general conditions of experiment. (2)	Meteorological data.						Difference of temperature between leaf and air, °C. (9)
		Percentage of sunshine recorded. (3)	Dry bulb thermometer. (4)	Wet bulb thermometer. (5)	Partial pressure of water-vapour in air in millimetres of mercury. (6)	Degree of humidity of air. Saturated air = 100. (7)	Velocity of wind in kilometres per hour. (8)	
			°	°				°
XIV. July 8, 1901	*Helianthus annuus*— Leaf A received full insolation, whilst B received exactly *one-half* of that incident on A	(A) 90 (B) 90	21 ·2 21 ·2	15 ·4 15 ·4	9 ·81 9 ·81	52 52	14 ·4 14 ·4	−0 ·17 −0 ·91
XV. July 9, 1901	*Helianthus annuus*— Leaf A in full sunlight .... Leaf B behind radial sectors arranged to cut off *one-half* of full radiation	(A) 100 (B) 100	22 ·2 22 ·2	15 ·1 15 ·1	9 ·13 9 ·13	46 46	8 ·0 8 ·0	−1 ·15 −1 ·46
XVI. July 11, 1901	*Helianthus annuus*— Leaf A in full sunlight .... Leaf B received *one-half* of solar radiation falling on A	(A) 100 (B) 100	27 ·2 27 ·2	19 ·0 19 ·0	11 ·5 11 ·5	43 43	12 ·0 12 ·0	−0 ·40 −1 ·84
XVII. July 15, 1901	*Helianthus annuus*— Leaf A received full insolation Leaf B received three-fourths of full insolation	(A) 70 (B) 70	22 ·2 22 ·2	15 ·6 15 ·6	9 ·60 9 ·60	48 48	17 ·1 17 ·1	−0 ·74 −0 ·97
XVIII. July 17, 1901	*Helianthus annuus*— Leaf A received full sunshine... Leaf B received three-fourths full sunshine	(A) 76 (B) 76	25 ·4 25 ·4	19 ·0 19 ·0	12 ·28 12 ·28	51 51	11 ·2 11 ·2	−0 ·82 −1 ·53
XIX. July 18, 1901	*Helianthus annuus*— Leaf A received full sunshine... Leaf B received seven-eighths full sunshine	(A) 100 (B) 100	27 ·5 27 ·5	19 ·7 19 ·7	12 ·26 12 ·26	45 45	18 ·2 18 ·2	−1 ·45 −1 ·55
XX. July 19, 1901	*Helianthus annuus*— Leaf A received full sunshine... Leaf B received seven-eighths full sunshine	(A) 75 (B) 75	27 ·6 27 ·6	19 ·6 19 ·6	12 ·03 12 ·03	43 43	11 ·7 11 ·7	−0 ·61 −0 ·84

Leaves and their Surroundings, the Incident Solar Radiation being Graduated Definite Amount of the Incident Radiation.

Estimated temperature of leaf. (10)	Excess of partial pressure of water-vapour in leaf over that of air in millimetres of mercury. (11)	*Assimilation* in cubic centimetres of CO_2 per square decimetre per hour. (12)	*Transpiration* in grammes of water per square decimetre per hour. (13)	Loss or gain of energy by the leaf in calories per square centimetre of leaf-lamina per minute.							
				R. Solar radiation incident on leaf. (14)	*a*. Coefficient of absorption. (15)	R*a*. Solar radiation absorbed by leaf. (16)	*w*. Energy expended in *assimilation*. (17)	W. Energy expended in *transpiration*. (18)	W + *w*. Total energy used for internal work. (19)	*r*. Energy lost by re-radiation and air-convection. (20)	(W + *w*) − R*a*. Energy received from surroundings. (21)
°											
21·0	8·65	*3·80	3·326	0·4547	0·686	0·3119	0·0031	0·3282	0·3313	—	0·0194
20·3	7·87	*3·80	2·580	0·2273	0·686	0·1559	0·0031	0·2548	0·2579	—	0·1020
21·0	9·33	*4·00	3·476	0·4798	0·686	0·3291	0·0033	0·3431	0·3464	—	0·0862
20·7	8·99	*4·00	2·740	0·2399	0·686	0·1645	0·0033	0·2704	0·2735	—	0·1090
26·8	14·66	*4·00	4·178	0·5492	0·686	0·3768	0·0033	0·4124	0·4157	—	0·0389
25·3	12·44	*4·00	3·715	0·2746	0·686	0·1884	0·0033	0·3668	0·3701	—	0·1817
21·4	9·32	*4·00	3·661	0·3931	0·686	0·2697	0·0033	0·3614	0·3647	—	0·0950
21·2	9·09	*4·00	3·270	0·2948	0·686	0·2022	0·0033	0·3229	0·3262	—	0·1240
24·6	10·68	*4·00	4·206	0·4993	0·686	0·3425	0·0033	0·4154	0·4187	—	0·0762
23·8	9·60	*4·00	4·014	0·3744	0·686	0·2569	0·0033	0·3964	0·3997	—	0·1428
26·0	12·69	*4·00	5·780	0·5504	0·686	0·3775	0·0033	0·5706	0·5739	—	0·1964
25·9	12·54	*4·00	5·438	0·4806	0·686	0·3298	0·0033	0·5368	0·5401	—	0·2103
27·0	14·44	*4·00	4·277	0·5337	0·686	0·3661	0·0033	0·4219	0·4252	—	0·0591
26·7	13·97	*4·00	4·040	0·4666	0·686	0·3201	0·0033	0·3981	0·4014	—	0·0813

Table X—

No. of experiment and date. (1)	Species of plant and general conditions of experiment. (2)	Meteorological data.						Difference of temperature between leaf and air, °C. (9)
		Percentage of sunshine recorded. (3)	Dry bulb thermometer. (4)	Wet bulb thermometer. (5)	Partial pressure of water-vapour in air in millimetres of mercury. (6)	Degree of humidity of air. Saturated air = 100. (7)	Velocity of wind in kilometres per hour. (8)	
XXI. July 24, 1901	*Helianthus annuus—*		°	°				°
	Leaf A received full solar radiation	(A) 13	17·4	16·2	12·89	87	20·9	+0·25
	Leaf B received five-eighths full solar radiation	(B) 13	17·4	16·2	12·89	87	20·9	+0·09
XXII. July 21, 1901	*Senecio grandifolius—*							
	Leaf A full solar radiation......	(A) 52	22·5	17·2	11·52	79	15·5	+0·96
	Leaf B *one-half* full solar radiation	(B) 52	22·5	17·2	11·52	79	15·5	−0·41
XXIII. June 24, 1901	*Senecio grandifolius—*							
	Leaf A full solar radiation......	(A) 66	18·3	11·4	6·89	44	18·9	+1·02
	Leaf B *one-half* full solar radiation	(B) 66	18·3	11·4	6·89	44	18·9	−0·02
XXIV. June 25, 1901	*Senecio grandifolius—*							
	Leaf A full solar radiation......	(A) 46	19·4	13·2	8·10	48	12·8	+0·40
	Leaf B *one-half* full solar radiation	(B) 46	19·4	13·2	8·10	48	12·8	−0·23

N.B.—In the case of experiments marked thus * the *continued.*

Estimated temperature of leaf. (10)	Excess of partial pressure of water-vapour in leaf over that of air in millimetres of mercury. (11)	*Assimilation* in cubic centimetres of CO_2 per square decimetre per hour. (12)	*Transpiration* in grammes of water per square decimetre per hour. (13)	Loss or gain of energy by the leaf in calories per square centimetre of leaf-lamina per minute.							
				R. Solar radiation incident on leaf. (14)	*a*. Coefficient of absorption. (15)	R*a*. Solar radiation absorbed by leaf. (16)	*w*. Energy expended in *assimilation*. (17)	W. Energy expended in *transpiration*. (18)	W + *w*. Total energy used for internal work. (19)	*r*. Energy lost by re-radiation and air-convection. (20)	(W + *w*) − R*a*. Energy received from surroundings. (21)
17 ·6°	2 ·06	*4 ·00	0 ·4096	0 ·1179	0 ·686	0 ·0808	0 ·0033	0 ·0404	0 ·0437	0 ·0371	—
17 ·5	1 ·96	*4 ·00	0 ·3303	0 ·0736	0 ·686	0 ·0505	0 ·0033	0 ·0333	0 ·0363	0 ·0139	—
23 ·5	9 ·97	*4 ·00	2 ·179	0 ·4288	0 ·774	0 ·3319	0 ·0033	0 ·2151	0 ·2184	0 ·1135	—
22 ·1	8 ·23	*4 ·00	1 ·763	0 ·2144	0 ·774	0 ·1659	0 ·0033	0 ·1736	0 ·1769	—	0 ·0485
19 ·3	9 ·73	*4 ·00	1 ·744	0 ·4080	0 ·774	0 ·3158	0 ·0033	0 ·1718	0 ·1751	0 ·1407	—
18 ·2	8 ·63	*4 ·00	1 ·599	0 ·2040	0 ·774	0 ·1579	0 ·0033	0 ·1576	0 ·1609	—	0 ·0030
19 ·8	9 ·05	*4 ·00	1 ·470	0 ·2468	0 ·774	0 ·1910	0 ·0033	0 ·1463	0 ·1496	0 ·0414	—
19 ·1	8 ·32	*4 ·00	1 ·183	0 ·1234	0 ·774	0 ·0955	0 ·0033	0 ·1167	0 ·1200	—	0 ·0245

values were estimated, not obtained by direct experiment.

Table XI.—Summarized Results of Table X of Experiments with Full and Partial Insolation with use of Radial Sectors. Disposal of Energy flowing into the Leaf when Total Energy received from Solar Radiation and Surroundings = 100.

	Expt. XIV.		Expt. XV.		Expt. XVI.		Expt. XVII.		Expt. XVIII.		Expt. XIX.	
	A. *Full* inso-lation.	B. *Half* inso-lation.	A. Fully inso-lated.	B. *Half* inso-lated.	A. Fully inso-lated.	B. *Half* inso-lated.	A. Fully inso-lated.	B. Three-fourths inso-lated.	A. Fully inso-lated.	B. Three-fourths inso-lated.	A. Full inso-lation.	B. Seven-eighths inso-lation.
w. Energy used for photosynthesis	0 ·65	0 ·94	0 ·66	0 ·94	0 ·56	0 ·72	0 ·67	0 ·78	0 ·57	0 ·63	0 ·44	0 ·47
W. ,, transpiration...	69 ·22	77 ·35	69 ·02	77 ·44	70 ·12	80 ·38	74 ·04	77 ·10	72 ·18	76 ·64	76 ·40	77 ·70
W + *w*. Total energy expended in internal work	69 ·87	78 ·29	69 ·68	78 ·38	70 ·68	81 ·10	74 ·71	77 ·88	72 ·75	77 ·27	76 ·84	78 ·17
R − R*a*. Solar radiant energy lost by transmission through leaf	30 ·13	21 ·71	31 ·32	21 ·62	29 ·32	18 ·90	25 ·29	22 ·12	27 ·25	22 ·73	23 ·16	21 ·83
r. Energy lost by re-radiation and air-convection	—	—	—	—	—	—	—	—	—	—	—	—
	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00	100 ·00

Table XI—*continued.*

	Expt. XX		Expt. XXI.		Expt. XXII.		Expt. XXIII.		Expt. XXIV.	
	A. Full insolation.	B. seven-eighths insolation.	A. Full insolation.	B. Five-eighths insolation.	A. Full insolation.	B. *Half* insolation.	A. Full insolation	B. *Half* insolation.	A. Fully insolated.	B. *Half* insolated.
w. Energy used for photosynthesis	0·55	0·60	2·79	4·48	0·76	1·46	0·80	1·59	1·33	2·23
W. " transpiration...	71·17	72·66	34·26	45·24	50·16	77·01	42·10	76·13	59·29	78·90
W + *w*. Total energy expended in internal work	71·72	73·26	37·05	49·72	50·92	78·47	42·90	77·72	60·62	81·13
R − R*a*. Solar radiant energy lost by transmission through leaf	28·28	26·74	31·40	31·38	22·59	21·53	22·59	22·28	22·60	18·84
r. Energy lost by re-radiation and air-convection	—	—	31·55	18·90	26·49	—	34·51	—	16·78	—
	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00

Jodrell Laboratory,
Royal Gardens, Kew.

On a New Method for the Determination of Atmospheric Carbon Dioxide, based on the Rate of its Absorption by a Free Surface of a Solution of Caustic Alkali.

By HORACE T. BROWN, F.R.S., and F. ESCOMBE.

(Received January 9,—Read March 23, 1905.)

In an appendix to a paper on the static diffusion of gases, communicated to the Society in 1900,* it was shown that when a current of air containing a constant proportion of carbon dioxide is caused to move in a turbulent stream over the free surface of a solution of caustic alkali, the rate of absorption of that gas increases with the velocity of the air-current up to a certain optimal speed, beyond which no further increase in the speed of the current influences the rate of absorption. It was further shown that when the optimal velocity of the air-current has been reached, and the temperature is maintained practically constant, the rate of absorption then varies directly as the partial pressure of the carbon dioxide in the air. In other words, if under the above conditions the rate of absorption per unit of area of the liquid surface is a for a partial pressure of carbon dioxide represented by p, and is a' for a partial pressure of p', then at similar temperatures, $a/p = a'/p'$.

A suggestion was also made that this principle might be found applicable to a determination of the carbon dioxide in air, and that if the method were found to be a practical one it would have the manifest advantage of not requiring any measurement of the air from which the gas was absorbed.

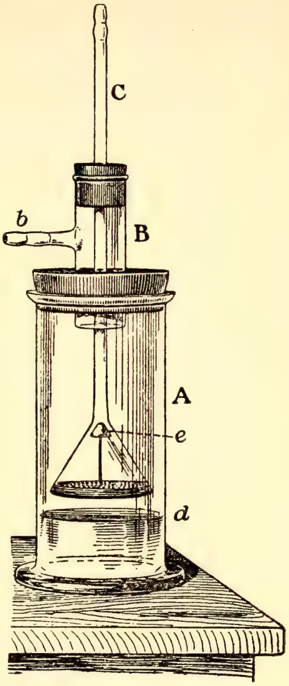
Since the first account of our experiments we have made a more complete investigation of the principles underlying the proposed method, and have succeeded in determining the coefficient of absorption of carbon dioxide under these conditions, and also the manner in which this coefficient is influenced by temperature. When certain precautions are taken, this simplified method of determining atmospheric carbon dioxide gives good results, which in point of accuracy approach those obtained by the more elaborate method with the Reiset's apparatus as described in the preceding paper.

The absorption-apparatus used in the experiments was a slightly modified form of one given at *b*, fig. 8 of 'Phil. Trans.,' B, Vol. 193, p. 284, which is here reproduced with its present modifications.

It consists of a glass cylinder A, about 15·5 cm. long and 6·5 cm. wide, closed with an india-rubber plug with a central perforation, through which passes a short wide tube B of 2·5 cm. diameter, opening out into the cylinder below, and having a side tube *b* which can be connected with the aspirator, or the aspirator and meter if measurements of the air are required. The upper part of the tube B is closed with a cork, through which passes a narrower tube C terminating below in a funnel, the width of which is only slightly less than that of the cylinder. The mouth of the funnel is closed with a perforated porcelain disc, which is luted in with paraffin. When air is aspirated through the side tube *b* it enters the apparatus down the narrow tube *c*, and passing through the perforated plate, impinges as a turbulent stream on the surface of the absorbing solution of caustic soda at *d*. In order to distribute the air more completely, a small cone of paraffin *e* is supported on a needle in the position shown in the Figure. The funnel can be adjusted to any desired distance from the surface of the liquid in the cylinder, the ordinary working distance being 1 cm. 100 c.c. of a solution of caustic soda of approximately 4 per cent. concentration are introduced into the cylinder through an india-rubber cap temporarily placed over its mouth, and a similar arrangement at the close of the experiment admits of the liquid being titrated without removal to another vessel.* At the point reached in the cylinder by 100 c.c. of liquid, the area exposed was 32·557 sq. cm.

---

* See 'Phil. Trans.,' B, vol. 193, p. 282.



Hart's method of double titration was used for determining the carbon dioxide absorbed by the alkali ; this has been already fully described.†

In determining the constants of the above apparatus the air-current employed was divided into two parts, one of which passed through the surface absorber, and the other through a Reiset's tower charged with the solution of caustic soda by means of which an independent and accurate determination of the carbon dioxide was obtained. In both cases the volumes of air passed were measured by standardized meters.

The first series of experiments, of which the results are given in the following Table, was undertaken for testing still further the accuracy of the above

---

* Care must be taken not to wet the sides of the cylinder above the level of the liquid.

† See 'Phil. Trans.,' B, vol. 193 (1900), p. 289; also 'Roy. Soc. Proc.,' this vol., p. 35.

Table I.—Showing the Relation of the Surface-absorption of Carbon Dioxide by a 4 per cent. NaHO Solution to the Partial Pressure of that Gas in the Air-Stream when the Velocity of the Air-Current exceeds the Optimum for Maximal Absorption.

N.B.—The experiment which is taken as unity for the ratios of absorptions and of partial pressure is one in which the CO_2 content is practically that of normal air.

1. No. of experiment.	2. Mean temperature, Centigrade.	3. Rate of flow of air-current through absorption apparatus. Litres per hour.	3. Mean linear velocity in metres per hour.	4. CO_2 in vols. per 10,000 of dry air determined by Reiset's apparatus.	5. Partial pressure of CO_2 in moist air in terms of $\frac{1}{10000}$ atmosphere.	6. CO_2 absorbed per hour by 32·557 sq. cm. of surface of NaOH (in cubic centimetres of CO_2 at N. T. P.).	7. CO_2 in cubic centimetres absorbed by 1 sq. cm. of surface per hour for one part in 10,000 of CO_2.	8. Ratios. (*a*) Of partial pressures of CO_2.	8. Ratios. (*b*) Of rates of absorption of CO_2.
1	14°·1	192·9	328	0·040	0·039	0·076	0·0583	0·012	0·013
2	12°·3	187·2	319	0·189	0·186	0·324	0·0526	0·056	0·058
3	14°·4	163·1	278	0·568	0·558	0·982	0·0531	0·168	0·177
4	14°·8	146·2	249	1·080	1·062	1·870	0·0531	0·320	0·337
5	12°·4	162·3	276	1·152	1·135	1·984	0·0553	0·342	0·357
6	11°·2	167·3	285	3·216	3·174	5·252	0·0501	0·958	0·946
7	13°·6	140·1	238	3·364	3·313	5·547	0·0506	1·000	1·000
8	15°·3	156·0	266	4·588	4·511	7·725	0·0517	1·361	1·392
9	13°·8	164·1	279	4·893	4·817	8·329	0·0522	1·453	1·501
10	13°·5	155·3	264	6·759	6·656	11·276	0·0512	2·009	2·032
11	13°·2	143·7	245	7·653	7·540	12·492	0·0501	2·275	2·250
12	13°·7	155·3	264	8·547	8·416	13·863	0·0498	2·540	2·499
13	13°·7	167·1	284	12·532	12·341	20·873	0·0511	3·724	3·763
14	15°·2	156·8	267	17·949	17·645	30·905	0·0528	5·325	5·571

proposition that under the conditions postulated, the rate of surface-absorption of carbon dioxide is proportional to the partial pressure of that gas in the moving air-current.

A preliminary series of experiments, which need not be quoted here, had already established the fact that with this special form of apparatus, and with amounts of carbon dioxide not exceeding about 14 parts per 10,000 of air, a velocity of current of about 150 litres per hour was sufficient to ensure a maximum rate of absorption. With the funnel adjusted to a distance of 1 cm. from the surface of the liquid, this corresponds to an average forward movement in the turbulent air-stream of about 260 metres per hour.

In those cases where it was required to employ air containing less than the normal amount of carbon dioxide, part of the air was previously passed through a tower containing soda-lime, the necessary admixture with ordinary air being made on emergence from the tower before it was divided between the two forms of apparatus. When, on the other hand, air richer in carbon dioxide than ordinary air was required, the air stream was passed through a tower containing fragments of marble, on which there dropped a graduated flow of one-tenth normal hydrochloric acid.

In the last two columns of the above Table are given side by side the ratios of the partial pressures of the carbon dioxide in the air employed, and the ratios of the rates of absorption by the surface of the alkaline solution. It will be seen that these series of values are practically identical, thus clearly showing the direct relation between the partial pressures and the rates of absorption.

The values given in the seventh column were obtained by dividing the number of cubic centimetres of carbon dioxide (measured at N. T. P.) absorbed per square centimetre of liquid surface per hour by the number of volumes of carbon dioxide contained in 10,000 volumes of dry air. The results represent the coefficient of absorption of carbon dioxide, stated in the form of cubic centimetres per square centimetre per hour, corresponding to a uniform partial pressure of 1/10000 of an atmosphere. Had there been an *exact* correspondence between partial pressure and rate of absorption in all these experiments, this coefficient would have been a constant. The variations which occur are largely due to differences of temperature ranging from 11°·2 to 15°·3 C.

In order to investigate the influence of temperature on the rate of surface-absorption of carbon dioxide by soda-solutions, a separate series of experiments was undertaken, in which the absorption cylinder was immersed in water, the temperature of which was observed at frequent intervals.* Six experiments of this nature were made between 13°·7 and 23°·7 C., ordinary air being used, of which the content of carbon dioxide was independently observed with the Reiset's apparatus.

Table II.—Showing the Influence of Temperature on the Coefficient of Absorption of CO_2 by the Surface of a 4 per cent. NaOH Solution.

Temperature.	Coefficient of absorption of CO_2 per square centimetre of liquid surface per hour, for 1 vol. of CO_2 in 10,000 vols. of air (dry).
13°·7	0·0519
15°·3	0·0537
15°·7	0·0537
19°·5	0·0583
21°·8	0·0623
23°·7	0·0635

On plotting out these results, it may be seen that within the experimental limits the coefficient of absorption varies directly with the temperature, and that an increase of temperature of 1° C. corresponds to an increase of 0·0018 in the absorption coefficient. Hence the value of this coefficient for any temperature t° within the above limits will be represented by $0{\cdot}0356 + t^\circ\, 0{\cdot}0018$, 0·0356 being the coefficient of absorption at 0° C., as determined by extra polation.

Assuming now that the relation of the partial pressures to absorptions is of the simple nature already postulated, then the volume of carbon dioxide contained in 10,000 volumes of dry air should be given by the formula

$$\frac{A}{0{\cdot}0356 + t^\circ \times 0{\cdot}00118},$$

where A represents the carbon dioxide absorbed per square centimetre of surface per hour, at temperature t°, from an air-stream which is drawn over the absorbing liquid at a sufficient rate to ensure the limit of maximal absorption being reached; A being stated in terms of cubic centimetres at normal temperature and pressure.

With the knowledge thus gained we are now in a position to compare the estimations of carbon dioxide made by means of the method of surface-absorption with those made with the Reiset's apparatus.

---

* In order to avoid evaporation and consequent cooling of the absorbing solution, the stream of air was previously saturated with moisture before being passed into the absorption apparatus.

In Table III we have taken the results obtained with the surface-absorption method as given in Table I, in which very variable amounts of carbon dioxide were present in the air-stream, and have calculated the amount present in volumes per 10,000 by means of the formula $\frac{A}{0{\cdot}0356 + t^\circ \times 0{\cdot}00118}$. Alongside these results we have given the amount of carbon dioxide found by an independent determination with the Reiset's apparatus.

Table III.—CO_2 in Parts per 10,000 of Dry Air.

No. of experiment.	By Reiset's apparatus.	By surface absorption with NaHO solution. $\frac{A}{0{\cdot}0356 + t^\circ \times 0{\cdot}00118}$.	Difference.
1	0·04	0·04	0·00
2	0·19	0·19	0·00
3	0·56	0·57	+0·01
4	1·08	1·08	0·00
5	1·15	1·21	+0·06
6	3·21	3·30	+0·09
7	3·36	3·30	−0·06
8	4·58	4·41	−0·17
9	4·89	4·93	+0·04
10	6·75	6·72	−0·03
11	7·65	7·50	−0·15
12	8·54	8·23	−0·31
13	12·53	12·40	−0·13
14	17·94	17·74	−0·20

A further comparative test of the two methods was made with ordinary air, and gave the following results:—

Table IV.—CO_2 in Parts per 10,000 of Dry Air.

No. of experiment.	Tempera-rure.	By Reiset's apparatus.	By surface absorption with NaHO. $\frac{A}{0{\cdot}0356 + t^\circ \times 0{\cdot}00118}$.	Difference.
1	15°·3	3·29	3·29	0·00
2	15°·7	3·33	3·32	−0·01
3	21°·8	3·05	3·10	+0·05
4	23°·7	3·15	3·16	+0·01

Jodrell Laboratory,
Royal Gardens, Kew.

On the Variations in the Amount of Carbon Dioxide in the Air of Kew during the Years 1898–1901.

By Horace T. Brown, F.R.S., and F. Escombe.

Received January 9,—Read March 23,, 1905.

As part of the routine work connected with our investigation of the processes of photosynthesis in plants, an account of which has been given in a previous series of papers, it became necessary from time to time to make a large number of determinations of the amount of carbon dioxide present in the air.

Since these determinations were made by a method which admits of a high degree of accuracy, and also extend over a considerable period, we have considered it desirable to collect the results, which are valuable in showing he limits of variation in the amount of this constituent of the atmosphere, of which a somewhat fallacious idea has been frequently entertained.

The method of experiment has already been described in a previous paper,* and it will, therefore, suffice to say that it consists in the absorption of the carbon dioxide from about 100 to 300 litres of air by means of a solution of sodium hydroxide contained in a Reiset's absorption apparatus, the amount of absorbed gas being subsequently determined by a process of double titration.

The experimental error incidental to the method falls well within + or −1 per cent. of the total amount of carbon dioxide estimated, so that the numbers representing the volumes of carbon dioxide per 10,000 volumes of air have certainly not a larger error than 0·03 parts per 10,000.

The air was taken from outside the Jodrell Laboratory in Kew Gardens, at a height of 4 feet 6 inches from the ground, and without any previous filtration.

The results, expressed in volumes of carbon dioxide per 10,000 volumes of dry air, have been thrown into a tabular form at the end of this paper, the date of each observation being given. (See Table II.)

The average value for the 91 experiments recorded is **2·94** volumes of carbon dioxide per 10,000 of air. The lowest value obtained (August 9, 1898) was **2·43**, and the highest (March 16, 1899) was **3·60** per 10,000. This latter result, obtained during a fog, was a very exceptional one, and out of the whole number of the 91 experiments recorded, in only nine did the carbon dioxide slightly exceed 3·20 parts per 10,000.

---

* 'Roy. Soc. Proc.,' this vol., p. 33.

As a general rule it would appear that the carbon dioxide present in the air is somewhat greater in the winter than in the summer months, but the year 1901 was an exception in this respect, since during July of that year it averaged 3·11 parts per 10,000. On the whole we are inclined to regard the periods of maximum carbon dioxide as being dependent more on anticyclonic than on seasonal conditions. The variations in the atmospheric carbon dioxide are small when regarded as absolute amounts, but they assume considerable importance in the light of what we now know about the relation of the partial pressure of the carbon dioxide in the air to the actual rate of assimilation by the green organs of plants.

In a previous communication* we have shown that under a given set of conditions favourable to photosynthesis a living chlorophyllous leaf assimilates from the surrounding air in a given time amounts of carbon dioxide which are *directly proportional to the partial pressure of that gas.* This is true up to limits of concentration of the carbon dioxide which lie altogether outside the variations which occur in Nature. Hence we have reason to expect that any well-marked variations in the carbon dioxide-content of the air during the active period of plant growth would produce some effect on the rate of nutrition of the plant.†

As a guide to the magnitude of the effects which may be expected from such variations as we are considering, we will take the mean carbon dioxide-content of the air for the month of July for each of the four years 1898 to 1901

---

* 'Roy. Soc. Proc.,' vol. 70 (1902), p. 398.

† This argument is not affected by the conclusion arrived at in a former paper ('Roy. Soc. Proc.,' vol. 70, p. 397) that although a proportionately increased intake of carbon dioxide takes place in a leaf when surrounded by an atmosphere containing three to four times the normal amount of carbon dioxide, yet the plant as a whole cannot avail itself of this increased amount of newly synthesized plastic material, owing to defective correlation of its various functions under these artificial conditions. Although a want of perfect adaptation of the plant in these respects undoubtedly exists in an atmosphere containing three or four times the normal amount of carbon dioxide, it by no means follows that the plant as a whole will not respond perfectly to the slight differences of carbon dioxide-content which occur under natural conditions.

Table I.—Mean CO_2 Content in Volumes per 10,000 Volumes of Dry Air, for the Month of July in each Year.

Year.	No. of determinations.	CO_2 in parts per 10,000.
1898	7	2·83
1899	9	2·88
1900	4	2·86
1901	7	3·11

We here see that in this month of active plant growth there was in 1901 an increase of 10 per cent. in the amount of atmospheric carbon dioxide over that of the three previous years. Assuming all other conditions to have been the same, both climatic and meteorological, such an increase would certainly be reflected in a correspondingly increased assimilation of the plant.

This example, and the results which are tabulated below, indicate that in the percentage variations of the atmospheric carbon dioxide we have factors which can no longer be neglected by meteorologists and agriculturists.

Systematic daily observations on the variations of the atmospheric carbon dioxide at widely separated stations are much needed for the purpose of ascertaining how far these variations are uniform over large areas, and also to what extent these changes in the composition of the atmosphere are reflected in the growth of our crops when they take place during periods of active plant growth.

Table II.—Carbon Dioxide in the Air of Kew expressed in Volumes per 10,000 Volumes of Dry Air.

Date.	CO_2 per 10,000.	Date.	CO_2 per 10,000.	Date.	CO_2 per 10,000.	Date.	CO_2 per 10,000.
1898—		1899—		1900—		1901—	
July 1	2·87	June 1	3·02	Aug. 22	2·74	Jan. 22	2·65
,, 6	2·77	July 4	2·84	,, 31	2·78	,, 29	3·03
,, 15	2·84	,, 7	2·99	Sept. 4	2·86	,, 31	3·18
,, 19	2·83	,, 12	2·82	,, 7	2·75	Feb. 18	3·36
,, 22	2·75	,, 14	2·92	,, 11	2·80	,, 21	3·21
,, 26	2·92	,, 18	2·80	,, 13	3·00	April 1	3·13
,, 29	2·83	,, 20	2·90	,, 18	2·91	,, 15	3·03
Aug. 9	2·43	,, 21	2·99	,, 20	2·71	,, 18	2·98
,, 31	2·84	,, 25	2·77	,, 25	2·85	,, 22	3·19
Nov. 29	3·18	,, 26	2·92	Nov. 4	2·73	,, 23	3·08
Dec. 1	3·06	Aug. 11	2·84	,, 17	2·65	May 1	3·29
,, 5	3·00	1900—		,, 20	2·93	,, 3	3·33
,, 13 (*a*)	3·23	May 29	2·93	Dec. 11	2·76	,, 13	3·09
,, 13 (*b*)	3·23	June 6	2·82	,, 12	2·75	,, 15	3·22
,, 16	3·00	,, 18	2·82	,, 13	2·81	June 27	3·04
,, 17	3·05	,, 22	2·84	,, 18	2·75	July 1	3·16
1899—		,, 26	2·84	,, 20	2·68	,, 4	3·08
Jan. 4	3·10	July 3	2·93	1901—		,, 9	3·20
,, 26	3·12	,, 5	2·86	Jan. 1	2·76	,, 11	3·15
,, 30	2·91	,, 11	2·84	,, 3	3·05†	,, 15	3·05
Mar. 16	3·60*	,, 18	2·83	,, 8	3·05‡	,, 22	2·93
,, 23	3·14	Aug. 7	2·71	,, 10	2·81	,, 24	3·22
,, 28	3·07	,, 11	2·79	,, 15	3·08§		
April 12	3·16	,, 17	2·82	,, 17	2·91‖	Mean	2·94

* March 16, 1899.—This is the mean of two concurrent experiments, giving 3·62 and 3·58 parts respectively. The result is exceptionally high and is doubtless connected with the thick fog which existed at the time.

† January 3, 1901.—Slight fog.

‡ January 8, 1901.—Foggy for several days preceding.

§ January 15, 1901.—Slight fog.

‖ January 17, 1901.—Dull with slight fog.

Jodrell Laboratory,
Royal Gardens, Kew.

On the Thermal Emissivity of a Green Leaf in Still and Moving Air.

By Horace T. Brown, LL.D., F.R.S., and W. E. Wilson, D.Sc., F.R.S.

Received January 9,—Read March 23, 1905.

(From the Physical Laboratory of the Daramona Observatory, Westmeath, Ireland.)

During the recent investigation of the energetics of a foliage leaf,[*] it was necessary to acquire some knowledge of the rate of interchange of heat between the leaf-lamina and its surroundings for a known excess of temperature, and under given conditions as regards the movement of the surrounding air. It was required, in fact, to determine with some approach to accuracy, the *thermal emissivity in air in absolute units,* including in this term the loss of heat due both to radiation and the conductive and convective properties of the surrounding air. The thermal emissivity is an important factor in the economy of the living plant, since it determines both the maximum temperature to which the leaf can be raised above its surroundings in those cases where the incident radiation is more than sufficient to perform the internal work of the leaf, and also the extent to which the leaf can be cooled below its surroundings when the receipt of radiant energy falls short of that required to produce the observed internal work.

There are comparatively few determinations in absolute units of the thermal emissivity of bodies cooling in air, and the results of experiments such as those of McFarlane on the cooling of a copper ball, or those of J. T. Bottomley, Schleiermacher, and Ayrton and Kilgour on the emissivity of platinum wires, cannot be rendered applicable to our requirements, owing in the first place to the nature of the emitting substances differing so widely from that of a leaf-lamina, and secondly to the fact, emphasized by the experiments of Ayrton and Kilgour,[†] that the loss of heat from radiation and air convection per square centimetre of surface per one degree excess of temperature is by no means constant, even for substances of a similar nature, and varies greatly with the size and shape of the cooling body.

In the absence of data from which we could deduce even the order of magnitude of the thermal emissivity of a leaf-lamina, it became necessary to attack the problem experimentally, and since the ordinary methods employed for determining the "rate of cooling" of a heated body are manifestly inapplicable to this particular case, we devised an indirect method which has given very good results, and is capable, we believe, of still further extension and refinement. It is based on the following principles:—

---

[*] See Brown and Escombe, 'Roy. Soc. Proc.,' this vol., p. 29.

[†] Phil. Trans.,' A, vol. 183, 1893, p. 371.

A leaf which is actively transpiring tends, by a process of self-cooling, to become lower in temperature than its surroundings. Assuming such a leaf to be placed in an enclosure, the walls and air of which are kept at constant temperature, whilst the air is maintained at a uniform state of humidity, the temperature of the leaf falls to a definite point below that of its surroundings, and then becomes stationary, providing the openings of the stomates do not alter. When this steady thermal condition is reached, the internal work of vaporisation manifestly becomes a measure of the amount of energy flowing into the leaf from its surroundings, providing we neglect, as we may safely do, the very small amount of heat self-produced in the leaf by the respiratory process.*

If, now, we have the means of determining, (1) the amount of water lost by the leaf in a given time by vaporisation; (2) the area of the leaf surface, and (3) the exact difference of temperature between the leaf-lamina and its surroundings, we have all the data necessary for ascertaining the amount of energy in water-gramme-units (calories) which flows into the leaf per unit-area of surface and unit-time for a 1° excess of temperature in the surroundings. But this is the same thing as the *thermal emissivity*, since emission and absorption are equal under the same relative conditions.

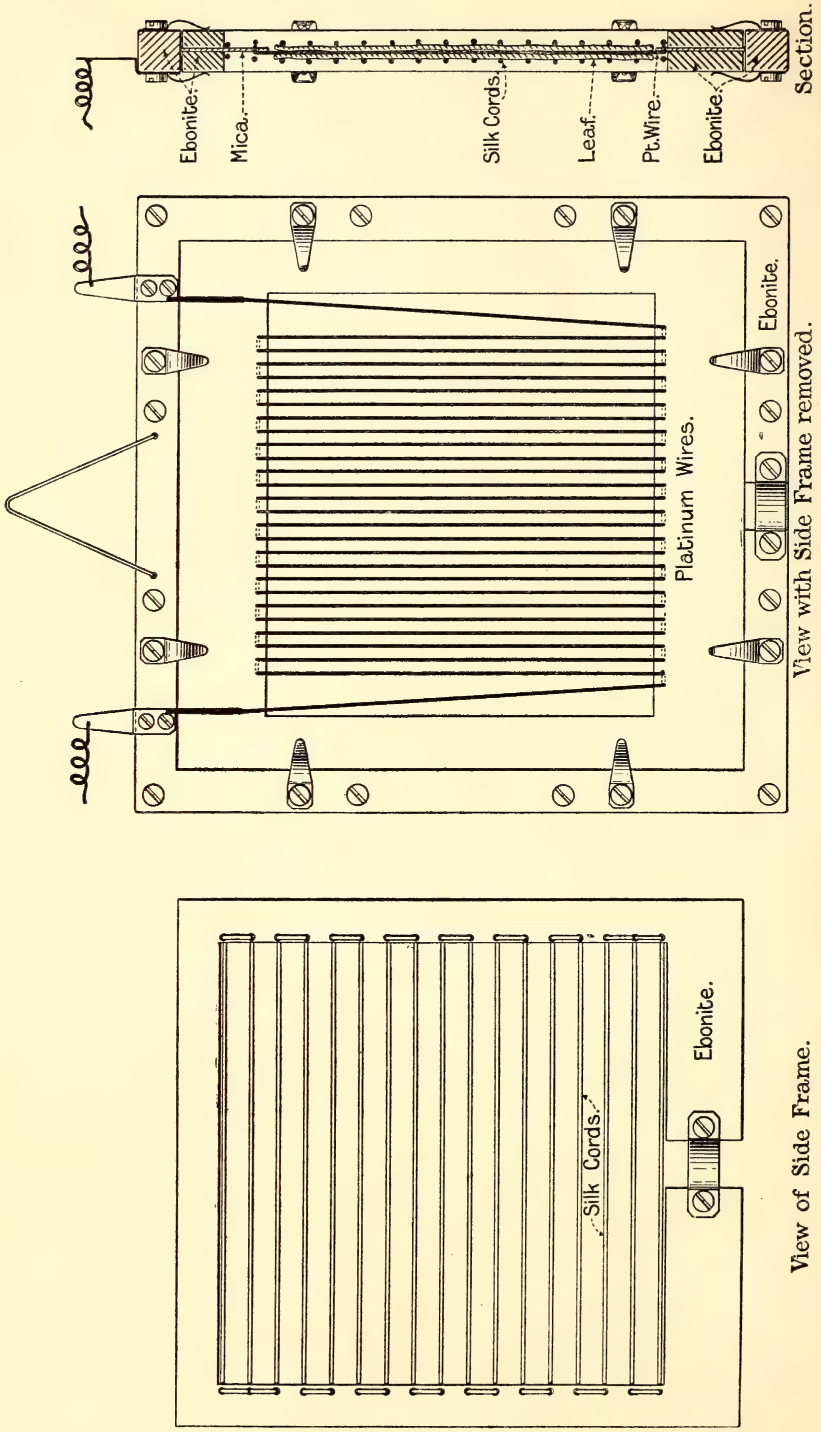
The range of temperature of any experiments of this nature is of course small, and it was therefore necessary to devise a form of apparatus, by means of which the temperature difference between the leaf and its environment could be accurately determined.

The thermometric apparatus used consisted of two differential platinum-resistance-thermometers, constructed for us by the Cambridge Instrument Co., and we must here express our great indebtedness to Professor Callendar for his help in designing the apparatus, and for most valuable assistance in other directions during the course of the investigation. The arrangement may be briefly described as follows:—

Two exactly similar frames of very thin ebonite, which in this particular instance had outside dimensions of 11·9 × 11·3 cm. and a width of 0·7 cm., were screwed tightly together on either side of a thin frame of mica which projected a few millimetres into the open part of the ebonite frame. This inner fringing edge of mica-plate was pierced top and bottom with a row of fine holes, through which was threaded a platinum wire of 0·006-inch gauge,

---

* *Cf.* Brown and Escombe, 'Roy. Soc. Proc.,' this vol., p. 71.


Ebonite.
Mica.
Silk Cords.
Leaf.
Pt.Wire.
Ebonite.
Platinum Wires.
Ebonite.
Silk Cords.
Ebonite.

Section.

View with Side Frame removed.

View of Side Frame.

Fig. 1.—Platinum Resistance Thermometer used for Determining Temperature of Leaves.

and of a length of about 2·4 metres, so arranged as to form a flat grid for the leaf-lamina to rest upon. The ends of the platinum coil were attached to two small brass lugs screwed on to the outer ebonite frame, and these could be connected with the leads of the recording instrument by suitable means.

Two other small movable frames of ebonite were constructed, which fitted accurately into either side of the outer frame, and rested, when in position, on the projecting edges of the mica-plate. Across these inner frames were laced thin silk threads for the purpose of lightly pressing the leaf-lamina against the platinum coil.

In using this instrument two similar leaves were always used, these being cut to the proper dimensions for placing in the outer frame. When the inner frames were brought into position, and fixed by means of small brass buttons attached to the outer frame, the platinum-resistance-coil was enclosed by the two leaf-laminæ in close apposition, and was very favourably placed for rapidly acquiring the mean temperature of the leaves.

Since the leaves had to be freely supplied with water, provision had to be made for their petioles to dip into small tubes of water, and for this purpose a portion of the lower part of each of the ebonite frames was cut away and bridged over with a thin curved piece of sheet brass of suitable dimensions. The leaf-stalks passed through a split cork which closed the mouth of the small water-tubes, and this was made tight with a mixture of paraffin and vaseline.* It will be noticed that the leaf surface exposed in this apparatus is always the same. The area of the free space of the inner frame was, in this particular case, 69·72 sq. cm., so that the total leaf surface exposed was 139·44 sq. cm.

The upper part of the outer frame was furnished with a loop of wire, by which the apparatus could be suspended to the arm of the balance when it had to be weighed.

Two exactly similar sets of frames were constructed, each of which, when mounted with their leaves and water-tubes, weighed about 60 grammes.

The difference in temperature between the two coils was determined by means of the Callendar recorder, and the resistance of the coils, which amounted to about 13·8 ohms at 18°·5 C., was very accurately adjusted for a "fundamental interval" of 5 ohms between 0° C. and 100° C., so that when a No. $\frac{1}{2}$ bridge wire was used in the recorder, each scale division of 4 mm.

---

* After the cork around the petiole has thus been rendered impervious by the wax, care has to taken to pierce this with a fine needle in order to allow the equalisation of pressure between the outer air and the air of the tube. If this precaution is not taken there is some danger of the free ascent of the water being impeded.

on the revolving drum was equivalent to a temperature difference between the coils of 0°·2 C.; differences of 0°·02 were readily measurable.

Our experiments were originally planned with the idea of determining the temperature-difference between the transpiring leaves and their environment by a comparison of the resistances of the platinum coils when one was clothed with its leaves and the other was exposed to free air. We have, however, to consider the possible effect produced by the heating of the coils during the passage of the current through them.

Mr. Francis Darwin, in a recent paper which came under our notice at the time we were planning these experiments,* has described the application of the platinum-resistance-thermometer and the Callendar recorder to a study of the leaf-temperature as an index of the condition of the stomata. With the small platinum coils he employed, which were wound on plates of talc 10 × 3 mm., it was found that the current used raised the temperature of the coils about 2° above the surrounding air, and that in order to avoid the difficulties introduced by inequalities in the rate of cooling, the central coil had to be covered with a "body equalling in volume and conductivity that on the experimental bulb."

In order to avoid as far as possible in our experiments the complications due to heating of the coils special precautions were taken in their construction, but the heating effect was still a sensible factor when using a current of 0·155 ampères. With a coil resistance of 13·8 ohms the heating effect of the above-mentioned current in each coil was $\frac{(\frac{10}{24}\mathrm{C})^2\mathrm{R}}{\mathrm{J}}$, *i.e.*, 0·01374 calorie per second, or 0·824 calorie per minute. The total surface area of a wire of 0·006-inch gauge and 2·4 metres in length is 11·491 sq. cm., so that the heat produced per square centimetre of surface per second is

$$\frac{0{\cdot}01374}{11{\cdot}491} = 0{\cdot}001195 \text{ calorie.}$$

From the experiments of Ayrton and Kilgour on the thermal emissivity of bright platinum wires of 0·006-inch diameter, we know that at 20° C. the emissivity is about 0·00222 calorie per square centimetre per second for a 1° excess of temperature.† Hence the maximum temperature to which the bare coil will be heated in "still air" by the above-mentioned current is $\frac{0{\cdot}001195}{0{\cdot}00222} = 0^\circ{\cdot}53$ C.‡

---

* 'Botanical Gazette,' vol. 37, 1904, p. 81.

† 'Phil. Trans.,' A, vol. 183, 1893, p. 371.

‡ Experimentally the temperature in still air was found to be 0°·54.

If we now suppose the other coil to be clothed with its leaves, the general effect of the heat produced by the current will be to reduce slightly the cooling effect due to transpiration, so that when the steady thermal condition is attained the leaf temperature will be somewhat higher than when no current is passing. If the rise of temperature of this whole system when the static point is reached is exactly equal to the rise of temperature of the bare coil, then the *temperature difference*, which is all that concerns us, will be the same as when no current is passing; in other words, we shall still have a measure of the differential temperature of the leaf and the surrounding air independent of the heating effect of the current.*

The whole question turns on the *thermal emissivity* of the leaf surfaces, which was shown by subsequent experiments to be about 0·00020 calorie per square centimetre of surface per second for a 1° temperature excess.

The total area of leaf surface exposed around the coil is 139·4 sq. cm., and the heat produced in the coil is, as we have seen, 0·01374 calorie per second; hence *when the steady thermal condition is reached*, the temperature of the system will be raised $\frac{0{\cdot}01374}{0{\cdot}0002 \times 139{\cdot}4} = 0^{\circ}{\cdot}49$.

This is only 0°·04 less than the temperature of the uncovered coil when it is in thermal equilibrium with its surroundings, so that the error introduced in determining the temperature difference between a transpiring leaf and its surroundings by comparison with a bare coil such as we used is small when the temperature difference measured amounts, as it frequently does, to 1° or 1°·5 C.

The method, in fact, works very well under perfectly still air conditions, and when precautions are taken to maintain the temperature of the enclosure perfectly constant; but very slight draughts of air or small sudden variations in the temperature of the air necessarily affect the bare coil much more readily than the leaf-covered coil, with the result (one to which Mr. Francis Darwin has already called attention) that the drum-record becomes very unsteady. In order to meet this difficulty we have adopted a modification of the method which is free from these objections, and which is entirely independent both of any heating of the leaf by the current used, and of the small amount of self-heating due to the respiratory process.

If the coils are covered each with a pair of similar leaves of exactly the same area, which differ only in their power of transpiration, and both pairs are placed in the same enclosure under exactly similar conditions as regards temperature and degree of movement of the air, then the difference in the amount of water transpired in a given time as determined by the balance will, when multiplied by the heat of vaporisation, give a measure of the excess number of calories entering the cooler leaf for a temperature gradient equal to the temperature difference given by the integrated record of the drum during the time occupied by the experiment.

---

* This argument is not in any way vitiated even if we suppose a portion of the heat of the leaf-clothed coil to be used in promoting increased transpiration.

In order to produce the differential transpiration on which the success of such experiments depends, advantage is taken of the fact that the stomata, through which the greater part of the vaporisation of the water takes place, are very seldom equally distributed on the two sides of the leaf. It is only necessary, therefore, to arrange one pair of leaves with their dorsal sides turned towards the platinum coils, and the other pair with their dorsal sides facing outwards. Hypostomatous leaves lend themselves best to such experiments, providing they are by nature good transpirers, since the dorsal side with its imperforate cuticle loses relatively little water compared with the ventral side.

Any changes in the dimensions of the stomata during an experiment have no influence on the final result, owing to the comparatively rapid thermal adjustments which take place in the leaf. Mr. Francis Darwin has already recorded the fact[*] which our experiments fully verify, that a slow and gradual closure of the stomata takes place when a cut leaf is placed in darkness. As the stomata close the record of differential temperature on the drum of the recorder approaches more closely the zero line of no temperature difference, but it is the *mean* value with which we alone have to deal in these experiments and, providing the record is correctly integrated, this mean temperature difference and the loss in weight of the two pairs of leaves respectively are the only experimental data required for determining the thermal emissivity of the leaf surface in absolute units.[†]

As an example of such an experiment performed under "still air" conditions, we may give the following. The leaf cases were placed in a covered tin case which was enclosed in an outer wooden case. A tray containing calcium chloride was placed at the bottom of the tin case in order to keep the air sufficiently dry to promote transpiration.

Experiment on the (hypostomatous) leaves of *Liriodendron tulipifera.*

Temperature of air in the enclosure 18°·6 C.

No. 1 *Coil.*—Leaves arranged with stomatiferous surfaces outwards.

---

[*] *Loc. cit.*

[†] Before each set of experiments it is always necessary to verify the zero line on the recording drum, since this is at times subject to small variations which are difficult to explain.

No. 2 *Coil.*—Leaves arranged with stomatiferous surfaces inwards.

Duration of experiment 2 h. 9 m. = 129 minutes.

Area of leaf surface exposed = 139·4 sq. cm.

Water lost by transpiration during experiment:—

No 1	0·640	gramme
„ 2	0·130	„
Difference	0·510	„

The mean temperature difference betwen the two pairs of leaves as indicated by the integration of the drum record was 7·05 scale divisions = 1°·41 C.

Since the difference in the amount of water vaporised by the two pairs of leaves is 0·510 gramme and the latent heat of vaporisation of water at 18°·6 C. is 593·6 water-gramme-units (calories), it follows that 0·510 × 593·6 = 302·7, represents in calories the excess of energy which must have entered the cooler pair of leaves from their surroundings during the experiment, an excess, which is conditioned solely by the temperature gradient of 1°·41 represented by the temperature difference between the two sets of leaves, for all other conditions are similar.

Since the surface area of the exposed leaves in each case is 139·4 sq. cm., and the time occupied by the experiment is 129 minutes, the *thermal emissivity* of this particular kind of leaf expressed in calories per square centimetre of surface per minute, per 1° C. temperature excess, is represented by

$$\frac{302 \cdot 7}{129 \times 139 \cdot 4 \times 1 \cdot 41} = 0 \cdot 01194 \text{ calorie,}$$

or 0·000199 calorie per second.*

---

* We have assumed that the thermal emissivity of the upper and lower surface of the leaf-lamina is identical.

Strictly speaking it is the *mean thermal emissivity* of the upper and lower surfaces which is measured by this method, but these mean results can be applied to the determinations of the emissivity of the entire leaf, including both sides, just as well as if we knew the respective emissivities of the two surfaces separately, since it is immaterial whether we take the total emissivity of the leaf as twice the mean value for the two sides, or as the sum of the separate emissivity-values, if these are known.

It is highly probable that even in glabrous leaves the emissivities of the upper and lower surfaces are not quite identical, for "surface emissivity" must depend to some extent on the *heat-conductivity* of the underlying tissue, even in a very thin lamina such as that of an ordinary foliage leaf. One would consequently expect that heat would flow into and out of the leaf somewhat more readily through the cuticle and epidermis overlying the more closely-packed palisade-parenchyma of the dorsal side, than through those portions overlying the spongy parenchyma of the ventral side with its numerous air-spaces.

We propose at some future time to investigate this question, which can be solved in the following manner: Having determined the thermal emissivity of two pairs of leaves, one of

The application of this method has been found to give very concordant results, and although the values have not, perhaps, the degree of precision required in the determination of physical constants, they are sufficiently close approximations to the true values to be exceedingly useful in any investigation of the energetics of the leaf.

It is scarcely to be expected that the thermal emissivities, even of individual leaves of the same plant, should be absolutely identical, and still more might we expect variations in leaves of dissimilar plants. Further investigation can alone decide the magnitude of these variations, but from the results we have obtained with the leaves of the four different species of plants mentioned below it is probable that leaves which are not highly glabrous, to which we have confined our attention, do not vary greatly in "emissivity."

Thermal Emissivity of Leaves of various Species of Plants under "Still Air" Conditions.

Species of Plant.	Thermal emissivity in calories per square centimetre of surface for a 1° C. temperature excess.	
	Per minute.	Per second.
Liriodendron tulipifera (1)	0·01194	0·000199
,, ,, (2)	0·01274	0·000212
Helianthus multiflorus	0·01499	0·000249
Tropæoleum majus	0·01427	0·000237
Tilia europœa	0·01598	0·000266

These emissivities were all determined within a range of temperature of from 17° to 19° C. The variation of the coefficient of emissivity with the temperature can safely be neglected for any range of temperature to which the leaf may be subjected under natural conditions.

It is worthy of note, in passing, that in their order of magnitude, the above values correspond to the emission of heat by a blackened copper sphere 2 cm. in diameter, cooling in air, as determined by McFarlane.* With a 5° temperature difference between the copper ball and its surroundings, he

---

which is arranged with the dorsal and the other with the ventral sides outwards, the experiment can be repeated after re-arranging the leaves on one coil so that one dorsal and one ventral side is exposed. From the difference, if any, in the emission-values so obtained it will be possible to ascertain the absolute emissivities of the upper and lower sides of the leaf respectively.

* 'Roy. Soc. Proc.,' vol. 20, 1871, p. 90.

found that the heat emitted per second, per 1° difference of temperature, per square centimetre of surface, was 0·000252 calorie.

The Thermal Emissivity of a Leaf in Moving Air.

In the experiments so far recorded, we have confined our attention to the "rate of cooling" of a leaf under "still air" conditions, that is to say, under conditions in which the leaf was shielded from direct draughts, and, as regards mass-movement of the air, was only subjected to the slight convective currents induced by the temperature differences between the leaves and their environment.

We have still to consider the influence of comparatively rapid air-currents of determinate velocity on the rate of transference of energy to and from the leaf, a matter of considerable importance in any study of the energetics of the leaf, especially as regards the dissipation of the excess of incident solar energy under open air conditions.

It has already been pointed out elsewhere* that, owing to the structure of the leaf, the rapidity of the transpiratory process, under fixed conditions of temperature and air-humidity, will not be much influenced by merely increasing the velocity of the air passing over the surface of the leaf when once a very moderate degree of velocity is exceeded. If, therefore, we have two pairs of leaves placed in the thermometric apparatus already described, and we arrange them so as to produce differential transpiration, the general tendency of a steady current of air passing over the leaves will be to diminish the temperature difference which they exhibit in still air conditions. Hence by extending our observations to leaves placed in air-currents of known velocity, we can determine the increase in the thermal emissivity due to this cause.

Before proceeding to describe our experiments in this direction we must refer to a previous paper which has a direct bearing on the subject.

So far as we know the only experiments which have been made on the influence of air-currents of definite velocity on the rate of cooling of a heated body are those of Crichton-Mitchell.† The author employed a blackened copper sphere which had a diameter of 2 inches, and the rate of cooling was determined in steady air-currents of determinate velocity. It was found that within limits of 200° C. temperature excess and a speed of air-current of 1000 metres per minute Newton's, "Law of Cooling" is accurate, provided the speed of the air-current passing the surface of the cooling body be sufficient.

---

* Brown and Escombe, 'Roy. Soc. Proc.,' this vol., p. 79.

† 'Roy. Soc. Edin. Trans.,' vol. 40, 1900, p. 39.

Moreover, and this is the point which is of particular interest for us, the cooling influence of the air is *directly proportional to its speed* up to velocities of about 300 metres per minute (18 kilometres per hour). Beyond this velocity of 300 metres the cooling influence in the case of the copper ball fell off somewhat. The explanation given, which is no doubt the correct one, is that when a body is cooling in air, the effect of radiation, which involves higher powers of the temperature excess (Stefan's "fourth-power law") is small compared with the effect produced by convection.*

Crichton-Mitchell's results are not expressed in absolute units, but from the data given it is possible to make a fairly close approximation to these. With a temperature difference of 10° C. between the copper ball and the air (the lowest experimental difference recorded) we have calculated (approximately) the calories lost per square centimetre of surface per minute for a 1° temperature excess for each of the given velocities of the air-current from 41 to 976 metres per minute. When the results were plotted the cooling effect of the air-current was seen to be practically a linear function of the speed up to velocities of 300 metres per minute, and that the extra cooling effect induced by the air-current amounted to 0·000206 calorie per square centimetre per minute per 1° C. excess, for an increased speed of the current of 1 metre per minute. But here again we are prevented from applying these values with any degree of certainty to the rate of cooling of a leaf owing to the different nature and shape of the cooling bodies. In the paper just cited the author proposed to apply his method of investigation to the cooling in moving air of a strip of platinum foil heated by means of an electric current, and it is probable that owing to the closer similarity in the shape of a platinum strip to the leaf-lamina such an experiment might give results which would have a more direct bearing on the special case we are considering, although there would still be the uncertainty due to the very different nature of the two laminæ.†

Our experiments on the influence of currents of air on the thermal emissivity of foliage leaves were carried out on lines very similar to those of Crichton-Mitchell, but owing to the differential method employed the apparatus admitted of simplification, the use of a water-jacket around the cooling body being unnecessary in our case.

---

* How relatively small a part radiation plays in the cooling of a heated body in air is shown by J. T. Bottomley's experiments ('Phil. Trans.,' A, vol. 178, 1887, p. 429) on the emissivity of heated platinum wires. From a series of experiments made in a high vacuum and in air at varying pressures it was found that only about 5 per cent. of the emissivity in air at 740 mm. was due to radiation.

† Professor Crichton-Mitchell has recently laid before the Royal Society of Edinburgh a preliminary account of his experiments on the cooling of a strip of platinum under the above conditions, but at the time of writing these results are not available.

In an aperture in one side of an air-tight box 3 feet cube was fixed an exhaust electric fan, 12 inches in diameter, and driven by an attached motor, which was connected with a speed-regulator under ready control. Into the side of the box opposite the fan opened a horizontal wooden shaft 5 feet in length. For the greater part of its length the cross section of this shaft was 10 inches square, but just before entering the box it was reduced to 5 inches, and at this point there was inserted an aluminium fan-anemometer constructed by Richard Frères. By means of this simple apparatus a steady current of air could be maintained in the horizontal shaft at determinate velocities up to about 140 metres per minute (8·4 kilometres per hour). The thermometric coils carrying the leaves were inserted through a small door in the middle of the shaft. They were placed at a sufficient distance apart to prevent any mutual disturbance, and with their planes parallel to the current of air which passed over the leaf surfaces.

The pairs of leaves covering the coils were arranged for differential transpiration in the manner already described, and a series of experiments was made for determining the thermal emissivity with air-currents of varying speeds on exactly the same lines as those for "still air" conditions.

The following results were obtained with leaves of *Liriodendron tulipifera*:—

Speed of air-current in metres per minute.	Thermal emissivity in calories per square centimetre of leaf surface per 1° C. temperature excess.	
	Per minute.	Per second.
"Still air"	0·0119	0·000198
36·2 metres	0·0173	0·000288
71·4 "	0·0238	0·000396
108 "	0·0304	0·000506
139 "	0·0361	0·000601

These results are plotted out in fig. 2, and clearly show that up to speeds of 140 metres per minute, the increased rate of cooling or heating of a leaf induced by a steadily moving current of air is directly proportional to the speed of the current passing over the surface of the lamina. This is quite in accordance with the general results obtained by Crichton-Mitchell, from which we are justified in concluding that the same law would hold good for even higher speeds, *i.e.*, up to at least 300 metres per minute (18 kilometres per hour).

The increase in thermal emissivity for a velocity of 1 metre per minute is seen to be 0·000174 calorie per square centimetre of leaf surface per minute per 1° C. difference of temperature between the leaf and the air.

Another similar experiment with the leaves of *Helianthus multiflorus*, having a thermal emissivity under "still air" conditions of 0·0150 calorie per square centimetre per minute per 1° C. excess temperature, showed an emissivity of 0·0338 calorie in an air-current of a speed of 109·8 metres per minute. This result is also plotted in the accompanying figure, and it will be seen that the line is sensibly parallel to that given by the experiments on *Liriodendron*, the rise of emissivity for an increased speed of 1 metre per minute being 0·000171 calorie, against 0·000174 calorie in the case of the *Liriodendron*. This renders it probable that although there are small initial differences in the emissivity of leaves under "still air" conditions, the influence of moving air-currents on all glabrous and moderately thin leaves in effecting transference of heat from and to the leaf is practically identical.

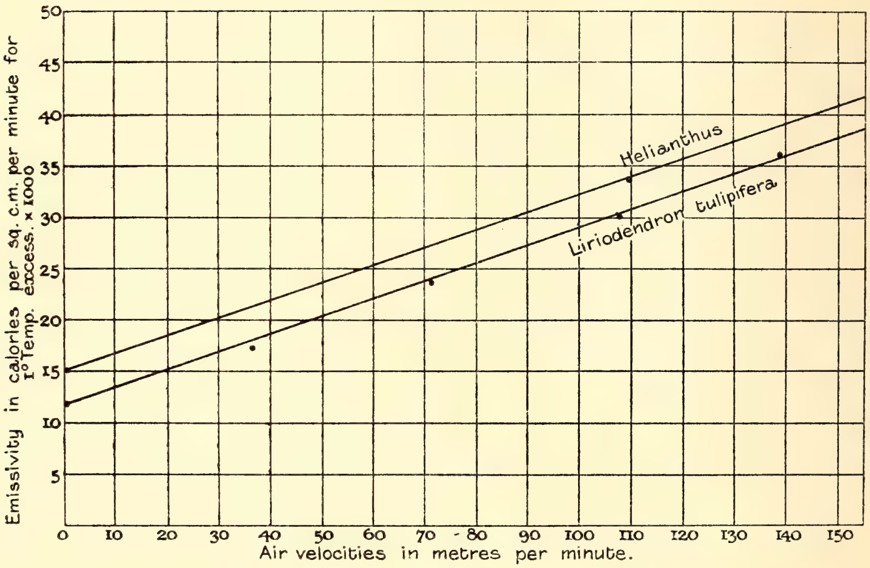


FIG. 2.

From the magnitude of the thermal effects produced by moving air it will be evident that this mode of dissipating the excess of radiant energy falling on the leaf must be of great importance, especially in those cases where the transpiratory work performed is small. Let us consider a somewhat extreme case in which the leaf is receiving solar radiation amounting to 1 calorie per square centimetre per minute, and let us assume that the absorption coefficient of the leaf for this radiation is 0·75, and that the leaf possesses a thermal emissivity equal to that of the *Liriodendron, i.e.*, 0·0119 calorie per square centimetre per minute for a temperature excess of 1° C. The total emissivity of the two surfaces of the leaf will, of course, be double this amount, *i.e.*, 0·0238 calorie per square centimetre per minute for a 1° excess.

If we assume transpiration to be entirely in abeyance, the temperature to which the leaf will be raised above the surrounding air when the emission exactly balances the absorbed radiation will be represented by $\frac{0\cdot75}{0\cdot0238} = 31^\circ\cdot5$ C., an excess which would speedily prove fatal to the leaf, even supposing the surrounding air to have a temperature as low as 20° C. If, however, we suppose the air to be in gentle movement at the rate of about 8·5 kilometres per hour (141 metres per minute), the emissivity of the leaf, counting both sides, becomes $0\cdot0361 \times 2 = 0\cdot0722$ calorie per square centimetre per minute for 1° of excess, and the leaf therefore cannot rise in temperature above the surrounding air more than $\frac{0\cdot75}{0\cdot0722} = 10^\circ\cdot3$ C., even when there is no dissipation of the absorbed energy by transpiration.

The importance of facts such as these in connection with the life-history of the xerophytic plants is considerable, and we are in a better position to give quantitative expression to these and similar problems connected with the energetics of the plant, now we have the means of determining the thermal emissivity of plant surfaces and the wastage of energy due to this cause.

We have shown above how it is possible to determine the thermal emissivity of a leaf both for "still air" conditions and for any given velocity of an air-current, provided we know the weight of water transpired per unit-area and unit-time, and also the temperature difference involved. Since, however, these three values representing thermal emissivity, water transpired, and temperature difference, are interdependent, it follows that when any two are known the third is calculable. Their relations may be conveniently generalized as follows:—

Let Q represent the amount in grammes of the water transpired per square centimetre of leaf-lamina per minute;

h, the latent heat of vaporisation of water at the temperature of the air* expressed in water-gramme-units;

$(\theta-\theta_n)$ the temperature difference observed; and

e the thermal emissivity of the leaf in calories per square centimetre of leaf surface, per minute, for a 1° C. temperature excess.

Then $$(1)\quad e=\frac{Qh}{(\theta-\theta_n)},\qquad (2)\quad Q=\frac{e(\theta-\theta_n)}{h},\qquad (3)\quad (\theta-\theta_n)=\frac{Qh}{e}.$$

Having once established and plotted the values of e for a particular kind of leaf, and for given conditions of air-movement it becomes possible by means of equation (2) to translate the temperature-records of the drum of the recorder, for any period, or for any particular moment of time, into *grammes of water transpired per unit-area, and unit-time.* If we are measuring the difference of temperature between the leaf and the surrounding air, it is the total loss of water by the leaf which is thus measured, whilst on the other hand, if both thermometer-coils are furnished with leaves, it is of course only the *differential transpiration-effect* which is determined.

As an example, we may take an experiment on the differential transpiration of two pairs of leaves of *Liriodendron tulipifera* in moving air, the details of which were as follows:—

Time occupied by the experiment, two hours.

Temperature of the air, 18°·5 C.

Latent heat of vaporisation (h) = 593·7 calories.

Mean differential temperature of the pairs of leaves $(\theta-\theta_n)=0°{\cdot}43$ C.

Velocity of air-current 71·4 metres per minute.

The thermal emissivity (e) of this leaf in an air-current of the above velocity is

$$e = 0{\cdot}0119+(0{\cdot}000174\times 71{\cdot}4) = 0{\cdot}0243.$$

From equation (2) the mean differential transpiration per square centimetre of leaf-lamina per minute, will be

$$Q=\frac{0{\cdot}0243\times 0{\cdot}43}{593{\cdot}7}=0{\cdot}0000176 \text{ gramme.}$$

This is equivalent to a differential transpiration of 0·1056 gramme *per square decimetre per hour.*

---

* The latent heat of vaporisation is represented by $606{\cdot}5-0{\cdot}695\,\theta$, where θ is the temperature of the air. Strictly speaking it is the temperature of the transpiring leaf which should be taken to represent θ, or, in the case of differential transpiration, the mean temperature of the two sets of leaves, but the error introduced from this cause is insignificant, amounting to not more than about 0·1 per cent.

The actual difference in the weight of water lost by the two leaf-cases during two hours as determined by the balance was 0·290 gramme, and since the total leaf surface exposed in each case was 139·4 square centimetres, the differential loss *per square decimetre per hour* was $\frac{0{\cdot}290 \times 100}{139{\cdot}4 \times 2} = 0{\cdot}1040$ gramme, against 0·1056 gramme, as determined from the differential temperature of the leaves.

It seems probable that this method of determining the rate of transpiration by a mere observation of temperature differences may be of service to those who are engaged in such investigations, since when once the thermal emissivity of the leaf is known, the position of the pen on the drum of the recorder enables us to determine at any desired moment the actual amount of transpiration which is going on in the leaf.

The actual difference in the weight of water lost by the two leaf-cases during two hours as determined by the balance was 0·290 gramme, and since the total leaf surface exposed in each case was 139·4 square centimetres, the differential loss *per square decimetre per hour* was $\frac{0{\cdot}290 \times 100}{139{\cdot}4 \times 2} = 0{\cdot}1040$ gramme, against 0·1056 gramme, as determined from the differential temperature of the leaves.

It seems probable that this method of determining the rate of transpiration by a mere observation of temperature differences may be of service to those who are engaged in such investigations, since when once the thermal emissivity of the leaf is known, the position of the pen on the drum of the recorder enables us to determine at any desired moment the actual amount of transpiration which is going on in the leaf.

On the Absence or Marked Diminution of Free Hydrochloric Acid in the Gastric Contents, in Malignant Disease of Organs other than the Stomach.

By Benjamin Moore, M.A., D.Sc., Johnston Professor of Bio-Chemistry in the University of Liverpool (in collaboration with W. Alexander, M.D., M.Ch., F.R.C.S., Hon. Surgeon, Royal Southern Hospital, Liverpool; R. E. Kelly, M.B., B.Ch., B.Sc., Alexander Fellow, University of Liverpool; and H. E. Roaf, M.B., Johnston Colonial Fellow, University of Liverpool).

(Communicated by Professor C. S. Sherrington, F.R.S. Received February 28,—Read March 16, 1905.)

It is well known that free hydrochloric acid is absent in a large proportion of cases of cancer of the stomach.

This was first noticed by Reinhard von den Velden* in 1879, and evoked a great deal of attention and experimental work, as a result of which some observers confirmed, while others disputed von den Velden's statement. But in the end the result that free hydrochloric is absent in the majority of cases of cancer of the stomach has become firmly established as one of the few experimental facts amid a mass of theory that we know regarding cancer.

It would be out of place in a preliminary paper such as the present to attempt a complete history of the enormous literature on the presence or absence of free hydrochloric acid in the stomach contents in cancer of that organ, so only a few of the prominent results recorded will be noticed as an introduction to the observations which form the subject of this communication.

In his original paper, von den Velden used methyl-violet as an indicator for free hydrochloric acid, and examined cases of dilatation of the stomach, due on the one hand to stenosis of the pyloric opening caused by carcinomatous growth, and on the other to various other causes. He found in ten cases of dilatation which were not due to carcinoma that free hydrochloric acid was always present, while in eight cases of cancer of the pylorus, free hydrochloric acid was uniformly absent, and he suggested such testing as a diagnostic sign for cancer of the stomach.

C. A. Ewald† raised objections to methyl-violet as not being a sufficiently sensitive indicator for free hydrochloric acid, and stated that in 23 cases of cancer of the stomach he obtained a clear reaction even with this indicator in 13 cases, a doubtful reaction in 5 cases, and no reaction in 5 cases.

---

* 'Deutsches Archiv f. klin. Medicin,' vol. 23, 1879, p. 31.

† 'Zeitsch. f. klin. Medicin,' vol. 1, 1880, p. 619.

In a reply to this, von den Velden* stated that he had only claimed, in the original paper, the absence of free hydrochloric acid in cancer of the stomach, leading to pyloric stenosis and accompanied by typical dilatation; and that he had stated that it still remained to investigate, as to the stage of the development of the disease at which the free hydrochloric acid disappears, if all forms of carcinoma of the stomach are alike in this respect, and if it makes any difference on which part of the stomach the growth is localized

Kredel,† in 17 cases of dilatation not due to carcinoma found free hydrochloric in every case, and in 19 cases of dilatation due to carcinoma that the free acid was invariably absent.

The next important contribution was made by F. Riegel,‡ who found in observations of the free hydrochloric acid repeated in the same cases over a period of several months, that von den Velden's rule was nearly always true. In a later paper Riegel stated that he found that more forms of gastric disease give an increase than a decrease in the amount of free hydrochloric acid, for example, in 128 cases, 19 showed an absence of free hydrochloric acid, 69 a hyperacidity, while the remainder gave an approximately normal amount of free hydrochloric acid. Of the 19 cases which showed absence of free hydrochloric acid, 16 were undoubtedly carcinoma, one amyloid degeneration of mucosa, and in one a backflow of bile was the cause.

A. Cahn and J. v. Mering§ as a result of finding free hydrochloric acid in about normal quantity in eight cases of *carcinoma ventriculi*, denied that absence is the rule. On the other hand, E. Korczynski and W. Jaworski‖ affirmed that for the great majority of cases the rule is absence of free hydrochloric acid.

The experimental methods of Cahn and v. Mering were subjected to adverse criticism by G. Honigmann and C. v. Noorden,¶ and their results shown to be due to organic acids being mistaken for free hydrochloric acid. In 14 cases of gastric cancer, Honigmann and v. Noorden showed that the acids present were chiefly organic, only traces of hydrochloric acid being present, and that free hydrochloric acid, added to the gastric contents in such cases of carcinomatous stomach dilatation, disappeared as such, being neutralized by salts of organic acids present, and setting free an equivalent amount of these organic acids.

---

* 'Deutsches Archiv f. klin. Medicin,' vol. 27, 1880, p. 186.

† 'Zeitsch. f. klin. Medicin,' vol. 7, 1884, p. 592.

‡ 'Deutsches Archiv f. klin. Medicin,' vol. 36, 1884, p. 100; 'Berl. klin. Wochensch.,' 1885, No. 9; 'Zeitsch. f. klin. Medicin,' vol. 12, 1887, p. 426.

§ 'Deutsches Archiv f. klin. Medicin,' vol. 39, 1886, p. 233.

‖ 'Deutsches med. Wochensh.,' 1886, Nos. 47 to 49.

¶ 'Zeitsch. f. klin. Medicin,' vol. 13, 1887, p. 87.

Further, these authors stated that they had observed cases of great dilatation, without carcinoma, accompanied by absence of absorption and retention of food in the stomach, as great as in the cases of carcinoma cited by them; yet in these cases of dilatation unaccompanied by carcinoma soon after ngestion of food there was plenty of free hydrochloric acid present.

In the literature subsequent to this period one finds chiefly records of a smaller number of cases, often of one case only, where regarded from the point of view of diagnosis, the absence of hydrochloric acid demonstrated the presence of malignant disease where the other signs were obscure; or, contrariwise, the presence of free hydrochloric acid was shown where there was undoubted malignant disease.

It is now well established that free hydrochloric acid is not absent in every case of cancer of the stomach, and also that it may be absent in other conditions than cancer, but the percentage of cases of cancer of the stomach in which it is absent is so large, that, taken in conjunction with other signs, it is a valuable aid in cases of doubtful nature. Thus Osler* records that in 94 cases in which the stomach contents were examined, in 84 free hydrochloric acid was absent; and v. Jaksch† in 17 cases found free hydrochloric acid, either absent, or only present in traces, in 14 cases.

The variations in results obtained by the earlier observers are due in part to the employment of different indicators of varying degrees of delicacy, and partly to the fact that the attention of these observers was turned almost wholly upon entire *absence* of the free acid as a diagnostic sign, so that little care was given to quantitative determinations of the variations in amount of the acid, or the relationship of this to the disease, which are far more important points.

If with the improved means for testing the matter both qualitatively and quantitatively now at our disposal, the extensive series of observations made by earlier workers were now repeated, it would probably be found, on the one hand, that in the long series of cases where the free acid was always found to be absent, there were in a certain number of cases traces present which were missed by the methods employed, and on the other hand, that in those series where in the majority of cases free hydrochloric acid was found to be present, the tests were given by larger amounts of organic acid, or the positive results were due to minimal amounts of free hydrochloric acid which were not determined quantitatively, and the small amount of free hydrochloric acid present was almost as good an indication of cancer as its entire absence. The entire absence of free hydrochloric acid is not in the nature of things to [be expected in every case, and the amount of such acid, as shown by quantitative tests, is the proper subject of enquiry both from the point of view of diagnosis and in regard to its relationship to the diseased condition itself.

---

* 'Principles and Practice of Medicine,' 3rd edition, p. 491.

† 'Klinische Diagnostik innerer Krankheiten,' 3te Auflage, p. 173.

Before coming to the proper subject of this paper, a little may be said with regard to the absence or diminution of the free hydrochloric acid in other forms of disease than cancer of the stomach. With the exception of atrophy of the gastric mucosa, where the gastric secretion and naturally the free acid is absent, there is no other diseased condition of the stomach in which the acid is absent in such a high percentage of cases as in cancer. Mörner* found in 12 cases of chronic gastritis that free hydrochloric acid was absent in two, and varied in amount in the other 10 from 0·02 to 0·12 per cent.

The free hydrochloric acid appears to be present in normal amount in phthisis, except in the last stages of the disease.†

It is often absent in acute infectious diseases, but much less commonly in other febrile conditions.‡ Other conditions in which absence has been noted are chronic kidney disease, anæmia, neurasthenia, hysteria, tabes, Addison's disease. It is also absent in prolonged inanition, so that in all cases the condition of the patient's appetite should be noted in applying tests for free hydrochloric acid.

Returning to diseases of the stomach in which the free hydrochloric acid is absent or greatly reduced in quantity, it may be stated that the conditions in addition to carcinoma in which the acid fails are those of atrophy of the gastric mucous membrane and chronic gastritis.

Now, in such cases, there is a very obvious and long continued perversion of the activity of the secreting cells, and, hence, it is not difficult to understand that continued local irritation on the one hand, or atrophy of the cells on the other, can lead to a suppression of the acid secreting function. But in cancer of the stomach the absence of free hydrochloric acid may be noted before the gastritis becomes chronic, and in cases where gastritis is not particularly marked. Also, complete suppression of the secretion of the acid may occur when only a small patch of the mucous membrane is involved by the disease, and where that portion is in the pyloric part in which normally no acid is secreted. The usual explanations of the absence of the acid in cancer of the stomach, viz., that the absence is due, as in chronic gastritis, to local irritation, or to neutralisation of the acid by alkali poured in from the ulcerating cancerous surface, seemed to me, therefore, scarcely to fit the facts of the case.

---

* 'Upsala Läkareförenings Förhandlingar,' vol. 24, 1889, p. 483; Maly's 'Jahresberichte ü. d. Fort. der Thierchemie,' vol. 19, 1890, p. 253.

† Immermann, 'Beiläge zu Centralblatt f. klin. Medicin,' vol. 10, 1889, p. 21.

‡ Gluzinski, 'Deutsches Archiv f. klin. Medicin,' vol. 42, 1888, p. 481.

That a small cancerous patch in one region of the stomach, occasionally so situated that it leads to no marked dilatation of the organ nor to any continued retention of food, should, *by some local irritation*, cause the suppression of the acid secretion in the remaining apparently healthy portions of the mucosa, did not seem to present a very feasible explanation. But if the suppression were not due to local action, to gastritis, or to the pathological condition of one portion of the mucosa affecting all the remaining portions, what could the explanation be?

The idea presented itself that the suppression of the acid might be due to a general condition in the body, to alterations of the circulating fluid in some way, either by products thrown out by the cancer cells, or as a result of changes in the blood which might lead both to the abnormal growth and atypical mitosis of the cancer cells, and to such changes in the nutrient medium of the oxyntic cells, that these could no longer separate hydrochloric acid from the inorganic constituents of the plasma.

Such a view, if it could be substantiated experimentally, would naturally give a new importance and a different aspect to what is already one of the most important experimental facts known about carcinoma.

The testing of this view was the object set forth in the observations recorded below, which have shown that *the absence of free hydrochloric acid in cancer of the stomach is not due to local action in that organ, for hydrochloric acid is absent or reduced greatly in amount whatever may be the situation in the body of the malignant growth.*

It follows that the absence of the acid is due to some change in the blood, which change may either be a common cause of the growth and the absence of the acid, or may be the result of the growth and the cause of the absence of the acid. The significance of this fact will be reverted to after the results of the observations have been described.

It is somewhat remarkable that no systematic observations have hitherto been made upon the condition of the gastric juice with regard to hydrochloric acid in malignant disease in other situations than the stomach, or if such observations have been made that they have excited so little attention. The literature on carcinoma is so enormous that it is possible such observations, although unknown to me, may have been made already, but such search as I have been able to make has revealed none, and if they do exist they have been awarded so little attention that they have been quite forgotten, and so it needs no excuse to publish the results of the observations given below.*

On placing the view outlined above before my colleague Dr. Alexander, he agreed to superintend the clinical side of the work.

The cases have been collected, under Dr. Alexander's directions, the administration of the test meals superintended, and the gastric contents obtained by Mr. Kelly.

The chemical analyses have been carried out in the bio-chemical laboratory of the university by Mr. Kelly, Mr. Roaf, and myself.

I desire here to express my thanks to my co-workers in the research, whose energetic co-operation has rendered the task of combining clinical and laboratory work an easy one, and also to those physicians and surgeons in Liverpool who have assisted us with further clinical material.

Methods of Examination.

The test meal given in each case was that recommended by Ewald of a pint of tea without sugar or milk, and a round of dry toast. Except in Case IX, where the toast could not be taken on account of the situation of the growth, and the test meal was a pint of gruel and two pints of water, administered by the stomach tube, and withdrawn after one and a-half hours. The length of time before withdrawal of the test meal is noted in each case, in the table.

---

* When the series of observations had been nearly completed, in a search through the literature in Maly's 'Jahresberichte über die Fortschritte der Thierchemie' (from which most of the quotations given in this paper have been cited on account of the inaccessibility to me at present of the original papers), I came upon a remarkable footnote by Maly to an abstract of the paper by Kredel, quoted above. The note occurs in vol. 14, p. 288, and is as follows :—"The fact that the portion of the mucosa of the stomach still intact secretes no hydrochloric acid is impossible to understand. If the carcinoma itself does not secrete an alkaline or neutralizing secretion, which ought to be observable in such new growths in other situations, a plasma richer in alkali must be considered as probable in cases of carcinoma. So that a failing acid formation may represent not so much a consequence as a cause or accompanying condition of cancer. Systematic investigation of urine, blood-serum, etc., in carcinomatous cases in regard to the alkali and acid relationships compared with those of normal individuals are accordingly much to be desired." It is curious that in spite of this view, which is practically the same as that which independently suggested the observations recorded in the present paper, no experiments were made in the direction suggested by such a distinguished physiological chemist as Maly, and that the matter should have been allowed to remain dormant for so many years.

The contents before testing were filtered from undigested residues of the food, and the tests in all cases were commenced as soon as possible afterwards.

The quantitative volumetric testing was performed by neutralising with decinormal caustic soda solution, with the indicator mentioned in each case. For convenience of comparison the results are expressed in terms of the equivalent amounts of hydrochloric acid. But it must, of course, be understood that in the case of the phenol-phthalëin, and di-methyl-amido-azo-benzol indicators, the figures do not represent actual free hydrochloric acid, but acidity expressed as the equivalent amount supposing it were all hydrochloric acid.

(a) *Total Acidity.*—This was determined in the usual way by titration of 10 c.c. of the gastric contents with phenol-phthalëin as indicator. The amount so obtained gives the total acidity due to hydrochloric acid (when present), organic acids, and acid salts (such as acid phosphates).

(b) *Acidity to "Di-methyl" Indicator.*—This indicator does not give as originally supposed by its introducer (Töpfer),* the free hydrochloric acid alone, but in addition, any acidity due to free, strong organic acids, such as acetic, lactic, and butyric. Accordingly, this figure is usually higher than that for the free hydrochloric acid given by the other methods described below, and the free hydrochloric acid may safely be taken as below the value of this reading. When the organic acids are low in value it gives an approximation to the free hydrochloric acid, and as it is often used as a clinical method, it is here given for purposes of comparison.

(c) *Acidity to Günzburg's Reagent*† (Phloroglucin and Vanillin).—We have found this reagent most reliable, both as a qualitative and a quantitative test. We have convinced ourselves by experiment that the test unmistakably shows 1 part of free hydrochloric acid in 30,000 parts by volume, and can be relied upon for accurate results in rapid clinical work where quantitative results are desired, as they always should be.

The use of the Günzburg reagent, in conjunction with titration with deci-normal alkali, which was first recommended by Mintz,‡ was carried out by us as follows:—

Ten c.c. of the filtered gastric contents are taken, two drops are removed with a glass rod to a porcelain capsule, a drop of the reagent added from a dropping bottle, and the mixture evaporated to dryness, preferably on a steam or water bath. If even a trace of free hydrochloric acid is present, the characteristic red colour appears. In that case, a quantity of deci-normal alkali is added to the 10 c.c. of filtered contents, from one- or two-tenths of a cubic centimetre to 1 c.c., according to the depth of colour obtained on the initial testing. The process of testing is then repeated, if a positive result is obtained more alkali is added, the testing repeated, and so on, until a negative result is obtained. Near the end, when the reaction is less marked, the alkali is added in quantities of 0·1 c.c. at a time. A little practice enables one to carry out the testing in about five minutes in all, and reduces the number of operations to four or five. As only about 0·1 c.c. is removed for each test, and the acid is almost neutralised when the final drops are removed, the loss in this way is very small.

---

* 'Zeitsch. f. physiol. Chem.,' vol. 19, 1894, p. 104.

† Günzburg, 'Chem. Centralblatt,' 1887, p. 1560; 'Centralblatt f. klin. Medicin,' 1887, No. 40.

‡ 'Wiener klin. Wochensch.,' 1889, No. 20; *ibid.*, 1891, No. 9. Maly's 'Jahresberichte ü. d. Fort. d. Thierchemie,' vol. 19, 1890, p. 255; *ibid.*, vol. 21, 1892, p. 222.

In many of the cases of malignant disease it will be observed that the test was negative from the outset, showing entire absence of free hydrochloric acid.

(d) *Modified Mörner-Sjöqvist Determinations*[*] *of Free and Combined Hydrochloric Acid.*—We have used this method as a gravimetric one in the modification described by v. Jaksch.[†] The method consists in converting all the acids present into barium salts by the addition of barium carbonate (previously tested, and found free from soluble barium salts). Ten c.c. of the gastric contents are taken,[‡] and about half-a-gramme of the fine dry barium carbonate powder added. The mixture is well shaken up and allowed to stand for about an hour, it is then evaporated down to dryness in a platinum or nickel crucible, and incinerated. In the process of incineration, the barium salts of the organic acids, if any are present, are destroyed, and barium carbonate is re-formed, while the barium chloride formed from the hydrochloric acid (free or combined with proteid) present in the gastric contents is unchanged in the process of incineration. After incineration, the incinerated mass is extracted several times with hot water, and the washings filtered free from barium carbonate.

The clear solution from the united washings measures approximately 50 c.c., when the washing is complete, and contains an amount of barium chloride which represents the total amount of free and combined hydrochloric acid in the original 10 c.c. of gastric contents.

The end of the process consists in determining the amount of barium chloride in the solution. Different volumetric methods have been proposed for this determination, but we have considered it more accurate to follow v. Jaksch's recommendation of weighing the precipitate.

The barium is precipitated in the usual way, at the boiling point, as sulphate by addition of a few drops of dilute sulphuric acid, kept at near 100° C. for about an hour in order that the precipitate may become granular, filtered through a Gooch crucible, dried and weighed.

---

[*] 'Zeitsch. f. physiol. Chem.,' vol. 13, 1889, p. 1.

[†] 'Monatsheft. f. Chemie,' vol. 10, 1889, p. 211; 'Klinische Diagnostik innerer Krankheiten,' 3te Auflage, 1892, p. 155.

[‡] Where previous titration in the malignant cases had shown that the total amount of acid was low, 20 to 50 c.c. were taken where available, so as to give a better chance of obtaining a figure for any minimal trace of hydrochloric acid which might be present.

From the weight of barium sulphate the amount of total hydrochloric acid (free and combined with organic matter) is then calculated.

In regard to this method, it must be stated that it gives not only the total amount of hydrochloric acid free or combined with proteid; but also the entire amount of inorganic acid (hydrochloric or other acid possessing a soluble barium salt) free or *combined with organic matter or a volatile base.*

The amount of such organic salts in normal stomach contents is small, but in the carcinoma cases the amount is probably larger. Thus in Cases XI, XVI, and XVII, the amount of hydrochloric acid given by this method is out of proportion both to the other cases and to the amounts of acid given by the other methods, this result can only be explained by the presence of compounds of inorganic acids probably hydrochloric, with volatile or organic bases, or with amido acids.*

This is further shown by the fact that in carrying out the incineration method for the determination of total organic acids,† although the titration figure with phenol-phthalëin is high, a negative result was obtained. In fact, of 10 c.c. of deci-normal alkali added in excess in Case XI, 6·6 c.c. disappeared, which could only arise from the presence in combination with inorganic acid of some organic base.

(e) *Total Free and Combined Organic Acids.*—An attempt was made to investigate this quantity by the following method. Ten c.c. of the filtered gastric contents are taken and titrated with phenol-phthalëin as in (a) for determination of total acidity. In this process as mentioned above, there are neutralised, the hydrochloric acid, the organic acids, and the acid salts (such as acid phosphates). The neutral solution is next evaporated to dryness, incinerated, taken up with hot water and boiled. In this process the sodium chloride formed from the hydrochloric acid remains unaltered, the neutral salts formed from the acid salts remain neutral; but the neutral organic salts formed from the organic acids, as well as any neutralised organic salts originally present in the gastric contents, are converted into carbonates and give an alkaline reaction. Accordingly the total organic acids present in the gastric contents, whether free or combined, are given, by now titrating the solution with excess of acid, boiling to remove carbonic acid, and back titration with deci-normal alkali.

The results, as given in the table, show that the amount of organic acids present, both in the normal controls and in the malignant cases, is very small, in fact, in some of the malignant cases, the results yield a negative value.‡ As indicated under the Mörner-Sjöqvist method this, however, indicates the presence of salts of organic bases with inorganic acids in the stomach contents, and hence, for both this method and the Mörner-Sjöqvist method it would be necessary to know the amount of organic bases present in order to obtain accurate results. The figures are given as of interest in showing that organic bases in combination with inorganic acid must be present in the stomach contents in these cases, and on account of the light they shed on the high result in Cases XI, XVI, and XVII with the Mörner-Sjöqvist method.

---

* Determinations in Case XI of the amount of ammonia by Schlössing's method showed that the amount of ammonia is small, so that the acid must be present in combination with organic bases.

† *Vide infra.*

‡ As shown by the fact that on incineration in the presence of excess of alkali, the amount of alkali recovered afterwards was less than that added in excess of amount necessary for neutralisation.

The presence under the pathological conditions of such compounds of organic bases with inorganic acid is in itself of high interest, and requires further investigation.

Determination of the Concentration of Hydrogen Ions by the Velocity of Inversion of Methyl-Acetate.

A determination of the concentration of the hydrogen ions in the gastric contents gives not only one of the best means of determining the character of the acid present, but furnishes the best guide to the real degree of acidity in the fluid.

The figures obtained by titration to the neutral point (or by a gravimetric method such as that of Mörner-Sjöqvist) give only what might be termed the total or potential acidity or alkalinity of a solution, and not the active acidity or alkalinity *at any given moment.* Thus, a deci-normal solution of any organic acid, such as acetic, lactic, or butyric, requires for titration to neutrality with a sensitive indicator such as phenol-phthalëin, just as much of a deci-normal alkali solution as does a deci-normal solution of an inorganic acid such as hydrochloric, sulphuric, or nitric, and, again, a solution of caustic alkali requires no more acid of any nature for neutralisation than does a solution of equi-normal strength of alkaline carbonate, or of an alkaloid. Yet we clearly recognise by the effects of such acids and alkalies that the state of affairs in such solutions are quite different, and that in popular language we have weak and strong acids and alkalies.

The explanation lies in the fact that only a certain fraction of the acid or alkali in the solution is effective at any given concentration, and the value of the effective fraction varies within wide limits with the nature of the particular acid or alkali in question.

When an acid is dissolved in water it becomes partially ionised into a hydrogen ion, and an ion represented by the remainder of the formula of the particular acid, while the remaining un-ionised portion maintains a balance or equilibrium in the solution, and is inert as regards acid properties, the degree of acid activity of the solution depending entirely upon the concentration of the hydrogen ions.

Similarly, when an alkali is dissolved, it becomes partially ionised into a hydroxyl ion, and another ion, dependent in constitution upon the alkali in question; and in part remains un-ionised. Here the hydroxyl ion, by its concentration determines the degree of activity as an alkali, just as in the case of the acid, the concentration of the hydrogen ion determines the acidity.

Taking, as examples, hydrochloric acid and potassium hydroxide, the condition of things when solution takes place may be represented by the equations:—

$$(m+n)\mathrm{HCl} = m\mathrm{HCl} + n\overset{+}{\mathrm{H}} + n\overset{-}{\mathrm{Cl}}$$

$$(m+n)\mathrm{KOH} = m\mathrm{KOH} + n\overset{+}{\mathrm{K}} + n\overset{-}{\mathrm{OH}}$$

The + and − signs indicate the ionic condition, and the fact that in electrolytic conduction the property of carrying the current lies in the ionised portions. Similarly, many other properties are due to the ionised condition, and amongst other, the acid and alkaline properties.

Now, for equilibrium in solution, a definite ratio must exist between m and n, which is dependent upon the nature of the acid or alkali and the concentration of the solution, and it is the variation of this ratio which is of interest to us here, and gives an experimental basis for determining the effective amount of acid or alkali in a given solution, which is not given by titration.

For example, hydrochloric acid in the neighbourhood of such concentrations as are found in the gastric contents is over 95 per cent. dissociated into its ions and is correspondingly effective as an acid, while acetic acid in similar concentration is only dissociated to the extent of about 3 per cent.* and is correspondingly weakened in its activity as an acid, and the same is true of all the other organic acids occasionally present in the gastric contents.

The same differences occur in the solutions of strong and weak alkalies, thus it was shown by Shields† that for dilute solutions of caustic soda and sodium carbonate of equi-molecular strength that the effective strength, or hydroxyl concentration, in the latter was only about 3 per cent. of the former. Such figures, as we shall see later, are of the utmost importance when we come to consider the relationship of the results we have obtained regarding the effective acidity of the gastric secretion to the effective alkalinity or acidity of the blood.

The blood owes its reaction to indicators (such as phenol-phthalëin, litmus, etc.) to such substances as sodium carbonate in presence of excess of carbonic acid, and to phosphates of the alkalies containing varying amounts of base and acid.

In such solutions the amount of effective acid ($\overset{+}{H}$ ion) or alkali ($\overset{-}{HO}$ ion) concentration is very low, and accordingly dependent upon the indicator used, the blood (and other fluids of the body, the various secretions, urine, milk, etc.) possesses an alkaline reaction (for example, to litmus or di-methyl) or an acid reaction (for example, to phenol-phthalëin). That is to say, in the case of the blood and many other fluids of the body there is both acidity and alkalinity according to the indicator used, for both acid ions and alkali ions are present, and the result obtained on testing will be dependent upon how the particular indicator is affected by these ions in the concentration in which they happen to be present.

In an analogous fashion to the different indicators, the different cells of the secreting and excreting glands of the body will separate from the same solution, the blood plasma, which bathes them, the ions for which they possess a greater permeability or greater affinity, and furnish secretions or excretions of varying reaction and degree of reaction, that is to say, of varying concentration in hydrogen and hydroxyl ions.

The varying reactions given with different indicators in the same fluid, looked at from the proper point of view, instead of being a source of confusion, hence serves to orientate us as to the cause of the reaction, and to furnish some conception of how acid or alkali can be secreted from the same common fluid.

---

* See Ostwald, 'Lehrbuch d. allgem. Chemie,' 2te Auflage, vol. 2, p. 729.

† 'Zeitsch. f. Physik. Chemie,' vol. 12, 1893, p. 167.

Now other things being equal in the way of permeability and affinity of the cell concerned in secretion, for the acid or alkali causing ions, it is evident that the power to secrete an acid or alkaline secretion will depend upon the concentration of these ions in the fluid which supplies the cell, that is the blood plasma.

But it must be carefully borne in mind that this factor cannot be obtained by titration of the plasma to neutrality in presence of an indicator. This does not give the effective concentration of the ions in solution, as is shown experimentally by the fact that the figure so obtained varies not only in amount but in algebraic sign with the indicator used, blood plasma being alkaline to "di-methyl," methyl orange, and litmus, and acid to phenol-phthalëin, and urine *alkaline* to the two former and acid to the two latter indicators.

The reason why such titration does not give the effective acidity or alkalinity is that an equilibrium is disturbed as the alkali or acid respectively are added.* Suppose, for example, that we are titrating the alkalinity of blood serum to litmus by means of a standard acid solution, then as the acid is added the hydroxyl ions are reduced by combining with the hydrogen ions of the added acid to form water. As a result equilibrium is disturbed, more hydroxyl ions are formed by breaking up of the undissociated carbonate or phosphate molecules to replace those used up, and the alkaline reaction persists until all the carbonate or alkaline phosphate has been used up.

Hence no knowledge is obtained by titration, of the real effective alkalinity at any instant, as determined by the hydroxyl ion concentration at that instant, but instead the titration figure gives the amount of acid necessary to reduce the bicarbonate and disodic phosphate to a certain condition with regard to the particular indicator used; that is, we do not get the value of the concentration of the hydroxyl ions when we began the titration, but instead, the amount of acid necessary to reduce the concentration of the hydroxyl ions to a dilution, at which the particular indicator in use is no longer affected, and this figure may bear no relationship to the concentration of hydroxyl ions, or effective alkali in the blood serum.

The consideration of this subject has been given at some length, because it affects not only the method we have here in view for determining the degree of effective acidity of the gastric contents, and incidentally the nature of the acid present, but casts a light upon the bearing of the absence or diminution of the hydrochloric acid on the probable changes in the blood giving rise to this condition.

---

* The formula for equilibrium is deduced as follows:—Suppose we have a solution represented by the equation $KOH = \overset{+}{K} + \overset{-}{OH}$, and let the concentrations of the molecules in solution be represented by C^{KOH}, C_K, and C_{OH}, then the tendency for K and OH to combine will evidently be proportional to the product $C_K \cdot C_{OH}$, and the tendency of KOH to split up into ions will be proportional to C_{KOH}. For equilibrium these two tendencies must balance, and hence $C_K \cdot C_{OH} = K \cdot C_{KOH}$, accordingly if one concentration varies such as that of C_{OH} on the addition of an acid, then the other concentrations must change correspondingly, that is, more KOH must dissociate.

It is clear that to obtain an estimate of the effective acidity or alkalinity of such solutions as occur in the body, we must make use of methods which do not disturb the equilibrium in the solution, and give us a measure from some activity of the hydrogen or hydroxyl ions. Such methods have already been elaborated in physical chemistry, and, at the suggestion of Ostwald, were first applied to estimation of the degree of acidity of the stomach contents by F. A. Hoffmann.*

The methods depend upon the fact that the velocity with which an acid solution hydrolyses a substance capable of hydrolysis, such as cane-sugar or methyl-acetate, is proportional to the concentration of the hydrogen ions in the solution.

For example, if cane-sugar solutions of equal strength be subjected to attack by equi-molecular solutions, on the one hand, say, of hydrochloric acid, and, on the other, of an organic acid, such as acetic. On account of the high state of ionisation of the hydrochloric acid, the cane-sugar is rapidly hydrolysed into invert sugar, and the rate of change can be accurately followed by the polarimeter, while, in the case of the acetic acid, which is but feebly ionised, the change is exceedingly slow. In fact, the change in the latter case is negligible compared with the former.

The application of the method with cane-sugar solutions and the polarimeter is, however, cumbrous, laborious, and slow, requiring a polarimeter, accurate readings, and the use of controls, since the gastric contents themselves rotate the plane of polarisation. Hence, the more recent method used by Hoffmann, which is an application of Ostwald's method of the catalysis of methyl-acetate, is much to be preferred, since it is much easier of application, can be applied in any laboratory where there is a thermostat or incubator, and facilities for volumetric analysis, and yields quite as accurate results as the other. The principle of the method is that methyl-acetate in aqueous solution changes with extreme slowness into methyl alcohol and acetic acid, according to the equation

$$CH_3.COO.CH_3 + H_2O = CH_3.COOH + CH_3.OH,$$

that this change can be increased enormously in velocity by the presence of an acid, and that the velocity is proportional to the concentration of the hydrogen ion of the acid, and the concentration of the methyl-acetate at any given moment.

The amount of acetic acid formed in a given time when acids of different concentration act upon the same concentration of methyl-acetate, gives, at once, an indication of the relative degrees of concentration of the hydrogen ions of the acids, or a simple calculation gives from this figure the effective concentration of the acid in hydrogen ions.

The determinations have been carried out by us as follows:—Ten c.c. of the gastric contents are taken in a small corked Erlenmeyer flask, 0·5 c.c. of the methyl acetate is added, and 5 c.c. of the mixture is titrated at once with decinormal alkali, free from carbonate, using phenol-phthalëin as indicator.

---

* 'Centralblatt f. klin. Medicin,' vol. 10, 1889, p. 793; 'Verhand. d. Internat. med. Congresses,' 1890, Abth. V, Abstract in Maly's 'Jahresber.,' vol. 21, 1892, p. 219.

The flask is then placed for a definite time in a thermostat at a temperature of 45° C. (we have used a period of eight hours, but a shorter period would suffice), and at the end of the period the contents of the flask are titrated again, the increase in the amount of alkali required for neutralisation gives the amount of acetic acid set free by the action of the hydrogen ions of the gastric contents during the interval, and hence an indication of the concentration of these ions. A simple calculation then gives the concentration of the hydrogen ions. For the purposes of this calculation, the total amount of acetic acid capable of being set free from the 0·5 c.c. of methyl-acetate added is required, and to obtain this a control is made in which deci-normal hydrochloric acid is allowed to act on methyl-acetate in the same concentration until the titration gives a constant figure for 5 c.c. of the mixture. This figure can be obtained once for all for any series carried out about the same time with the same sample of methyl-acetate.*

A comparison of the results given in the table of the titrations for acetic acid, formed from the methyl-acetate in the cases of malignant disease, as compared with those in the case of the normal control specimens, demonstrates clearly the value of the method, and shows the great contrast between the cases of malignant disease and the others.

The equation giving the velocity constant of the reaction (K), which is proportional in each case to the concentration of the hydrogen ions, takes the form—

$$K = \frac{1}{t} \cdot \log \frac{A}{A - x},$$

where t is the time, expressed in minutes, A is the amount of methyl-acetate available for hydrolysis in the beginning, and x the amount of methyl-acetate hydrolysed at the end of the time t. The column in the table gives the values of $K \times 10^5$, to which the concentrations of hydrogen ions, or the effective acidities are proportional, and the last column gives the percentage concentration reckoned as effective hydrochloric acid, by comparison with the constant for a deci-normal solution (0·365 per cent.) of hydrochloric acid.

Deductions from the Results given in the Table.

1. The *total acidity* in malignant disease, wherever situated, is, as a rule, very low. In the 17 cases recorded, the total acidity only rises above 0·1 per cent. in four cases (Nos. V, VI, XI, XVI), and reaches the normal amount of approximately 0·28 per cent. in one case only (No. XVI). In the great majority of the cases one or two drops of deci-normal alkali, added to 10 c.c., is sufficient to render the reaction alkaline to phenol-phthelëin. In the few cases where the total acidity rises above a trace only, the other tests

---

* The methyl-acetate should be as pure as possible, give practically no acetic acid on titration alone, and a control as indicated above should be carried out at intervals, if the series of experiments is a prolonged one.

Table of Amount of Acidity of Gastric Contents in Cases of Malignant

No. of case.	Sex.	Age.	Disease and region.	Period of digestion.	Total acidity to phenol-phthalëin.	Acidity to di-methyl.	Free hydro-chloric, Günzburg.
I	F.	49	Carcinoma of uterus	1 hour	0·0219	Negative	Negative
II	M.	—	Carcinoma of prostate	,,	—	—	,,
III	F.	—	Sarcoma, general, liver, etc.	1¼ hours	0·0146	Negative	,,
IV	F.	71	Carcinoma of uterus	,,	0·0036	,,	,,
V	F.	50	,, ,,	1 hour	0·1460	0·0821	0·0365
VI	M.	61	Sarcoma of neck	,,	0·1861	0·0036	0·0072
VII	F.	65	Carcinoma of liver	1¼ hours	0·0018	Negative	Negative
VIII	F.	32	Carcinoma of rectum	1 hour	0·0584	,,	,,
IX	F.	—	Carcinoma of tongue	1½ hours	0·0292	0·0186	0·0109
X	M.	61	Epithelioma of floor of mouth	1 hour	0·0018	Negative	Negative
XI	F.	49	Colloid cancer of mesentery	1¼ hours	0·1058	0·0401	0·0072
XII	F.	59	Carcinoma of breast, removed 10 weeks previously	1½ hours	0·0182	0·0091	0·0036
XIII	F.	59	Recurrence of carcinoma in breast, removed 4 years before	,,	0·0511	0·0219	0·0036
XIV	M.	65	Epithelioma of cheek	1 hour	0·0018	Negative	Negative
XV	F.	66	Carcinoma of breast	,,	0·0548	0·0019	,,
XVI (*a*)	M.	63	Epithelioma of cheek	3 hours	0·2665	Negative	,,
(*b*)				1 hour	0·2847	,,	,,
XVII	M.	57	Recurrence of carcinoma of tongue, removed 6 years ago	2 hours	0·0365	,,	,,
							Control Cases in
R. E. K.	M.	25	—	1 hour	0·2117	0·1789	0·1533
B. M.	M.	38	—	,,	0·3285	0·2884	0·2519
H. E. R.	M.	23	—	,,	0·3139	0·2482	0·1862
Average	—	—	—	,,	0·2847	0·2385	0·1971
N/10 HCl	—	—	—	—	—	—	—

---

* See text, pp. 146–7. † Total amount hydrolysed in 24 hours, 31·6 c.c.;

Disease in Different Situations as determined by Methods indicated.

Total hydrochloric, *i.e.*, free and organic combined, Mörner and Sjöqvist.	Alkali after incineration, reckoned as HCl.	Increase in titration in cubic centimetres after hydrolysis of methyl-acetate.			Constant of velocity of reaction, proportional to real acidity, as shown by concentration of hydrogen ions, K × 10^5.	Effective acidity reckoned as percentage of HCl from velocity constant.
		Initial.	Final.	Increase.		
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	0·0329	—	—	—	—	—
—	0·0066	—	—	—	—	—
—	0·0146	—	—	—	—	—
—	0·1533	—	—	—	—	—
—	0·0000	—	—	—	—	—
0·0013	0·0036	0·8	0·95	0·15	1·1649	0·00100
0·0295	0·0000	0·4	2·2	1·8	14·429	0·01239
Unweighable	0·0000	0·1	0·1	0·0	0·000	0·00000
0·0738(?)*	−0·1350*	1·55	3·65	2·1	16·934	0·01455
0·0023	0·0000	0·25	1·30	1·05	8·295	0·00713
—	0·0000	0·85	2·10	1·25	9·913	0·00852
0·0044	0·0000	0·025	0·05	0·025	0·1937	0·00017
0·0020	−0·0402*	0·8	1·05	0·25	1·945	0·00179
—	0·1533	2·7	2·8	0·1	0·7759	0·00067
0·1479(?)*	0·2044	2·7	2·7	0·0	0·0000	0·00000
0·1140(?)*	−0·0657*	0·4	0·4	0·0	0·0000	0·00000
Normal Individuals.						
0·1579	0·0018	3·05	17·9	14·85	167·300	0·14373
0·2571	0·0000	4·8	25·5	20·7	305·740	0·26266
0·3296(?)*	−0·0876*	4·0	21·1	17·1	208·248	0·17892
0·2482	—	—	—	—	227·096	0·1951
—	—	4·8†	28·2†	23·4†	424·870	—

in 48 hours 31·7 c.c., leaving 26·9 as total available amount of acetate hydrolysable.

demonstrate that the reaction is not due to free hydrochloric except in minute traces.

2. The "di-methyl" indicator shows entire absence of acidity in nine out of 16 cases in which it was applied, and in the remaining cases gives very low values, approaching half-way towards normal in only one case (No. V).

3. The Günzburg test shows entire absence of free hydrochloric acid in 11 out of 17 cases, and in the remaining cases the quantitative use of the test shows that, with one exception (Case V), the amount of hydrochloric acid present was only a minute trace (0·0036 to 0·0109 per cent.). Case V was the only one which had an appreciable amount of free hydrochloric acid, and even here the amount present was less than one-fifth the normal quantity.

4. The Mörner-Sjöqvist method gave, as a rule, low results, but in Cases XI, XVI, XVII much higher results were given than by the other methods. There is little doubt that this result arose in those cases from the presence of salts of inorganic acids with organic bases, and not to hydrochloric acid either free or loosely combined with proteïd.

5. The presence of inorganic acid combined with organic base is shown by the zero or negative value obtained on incineration with excess of alkali in the attempt to determine the amount of organic acid.

6. The methyl-acetate inversion method shows clearly what small traces of effective acid are present as compared with the normal cases. In all the cases tested the concentration of hydrogen ions never exceeds one-fifteenth part of the normal amount, and in the majority of cases sinks incomparably lower even than this low fraction.

7. Attention may be drawn to Case XII, in which a carcinomatous breast had been removed ten weeks previously, recovery was completed and appetite good. Here it is to be noted that the gastric contents possess scarcely any "total acidity," requiring only 0·5 c.c. in 10 c.c. of deci-normal alkali for neutralisation, and that of this trifling amount only one-fifth or less (0·1 c.c. N/10 in 10 c.c. of the contents) is shown to be free hydrochloric acid by the Günzburg test.

This shows, so far as any conclusion can be drawn from a single case, that the suppression of acid is not due to secondary products thrown out by the growth, neutralising the acid ions of the plasma. For in that case the acid secretion should be re-established after removal of the growth. The persistence of absence of acid secretion after the growth has been removed points to the view that the condition of the blood, and most probably the absence or marked diminution of acid ions in it, is to be regarded as a cause predisposing to growth formation, and not as an effect of the growth. In this case there persist, even after the growth has gone, the same factors, as indicated by the absence of the acid, which initially lead to the growth occurring, and the patient, on account of this condition, lies open to the risks of a recurrence.

It is clear that the study of the condition of the gastric contents with regard to acid subsequent to operation carried out in a large number of cases, must cast interesting light upon the problem before us, and we are now attempting to obtain as many as possible such cases.

The results of Case XIII, in which recurrence had just begun to be obvious, are also interesting from this point of view.

8. In the table are included two cases of sarcoma, in which similar results were found as in carcinoma. It was at Mr. Kelly's suggestion that such cases were included, and we are at present in the position of waiting for further material, but if the results in these two cases are confirmed in others, an interesting parallelism between the two types of malignancy will have been established, pointing to something very common in mode of origin of malignant tumours.

Discussion of Results.

1. The importance of the marked depression or entire suppression of the acid-secreting function, no matter what the situation of the growth, as an aid to diagnosis in doubtful cases of cancer, need not be insisted upon.

It must, however, be pointed out that we have not up till the present been regarding the subject primarily from that point of view, and hence most of our cases were well advanced. Accordingly, observations are still required at early stages in the disease, before conclusions can be drawn as to the diagnostic value of the sign, and such observations should be quantitatively directed towards determining amount of free hydrochloric acid, and not merely its qualitative presence or absence, as is too often done.

2. The bearing of the results upon the cause and possible prevention of the malignant growths is the most important aspect to be considered. Instead of regarding absence or diminution of hydrochloric acid in cases of cancer of the stomach as being due to some local effect in that organ, we obtain from the observations recorded above the information that both in stomach cases and all other cases the change in acid secretion is not due to local influence on the secreting cells, but to an altered condition of the blood. That the reduction of acid-secreting power is a general effect accompanying malignancy wherever the growth may occur in the body.

So far, we are dealing with experimental fact, and not with theory or hypothesis, and continued observations of the acid-secreting function under varying conditions (such as early in the disease, soon after removal of growth, repeated observations at varying intervals after operation, and observations early in recurrence), must teach us the relationships of the variations in acid production to the appearance of new growth.

It may be allowable, however, in concluding this paper, to throw out a few suggestions, as to the probable cause of the change in the secretion of the acid, which are capable of forming the basis for additional investigation.

(*a*) The diminution in acid secretion may be due to atrophy or loss of function of the oxyntic or acid-secreting cells.

A diminution in the percentage of hydrochloric acid has been observed in old and senile individuals, the average amount of free hydrochloric acid being, it is said, only slightly over 0·1 per cent. instead of 0·2 per cent. This diminution is, of course, very much less than in the malignant cases, but it shows that there is a tendency to diminution of acid-secreting power in the cells with advancing age, and hence suggests that in carcinomatous cases there may be an abnormal diminution in this power of the cells to secrete acid.

Accordingly an investigation of the histology of the gastric mucous membrane is suggested in cases of malignant disease, especially where the growth has not invaded the stomach. Such investigation would have to be done most carefully, because long continued perversion of function might lead to atrophy as a secondary and not a primary effect. Also *post-mortem* work alone might easily lead to wrong results, because the gastric cells must share in the general wasting due to the growth. The experiments indicated might hence best be taken up in the case of animals suffering from malignant disease.

(*b*) The diminished acid secretion may arise from changes in the circulating medium, which do not cause atrophy of the cells, but which alter the activities of the cell so that it can no longer form acid; or the materials for the formation of the acid may be lacking in the material supplied to the cell.

First, toxic substances may be formed at the seat of growth which circulate to the oxyntic cell and pervert its functions, alter its permeabilities for different ions, or in some manner destroy its normal power of secreting the acid. In this case if the growth were early removed, before the oxyntic cells had been long thrown out of normal action, then one would expect a return to the normal condition of affairs. Accordingly, we here require systematic and careful investigation of the effects of early removal of the growth upon the acid secretion

Secondly, the action may not be a toxic one in the above sense, but may be due to the absence or marked diminution of the sources from which the cell prepares the acid in the plasma supplied to it.

In such a case there would not be a rebound towards normal acidity on removal of the growth, the sub-acidity would remain, and also the condition of which it is the reflex would remain in the blood, and favour a reappearance of new growth wherever in the body there was a tendency for the cells to take on the abnormal type of reproduction. Let us consider briefly the conditions under which the cell forms free hydrochloric acid from the blood plasma, apart from theories of an older date as to particular salts between which hypothetical reactions were regarded as taking place.

The end result of the normal secretory activity of the oxyntic cell is the production from the blood plasma of an acid solution containing 0·2 to 0·3 per cent. of free hydrochloric acid. That is to say, a solution has been produced containing hydrogen and chlorine ions in a certain concentration. Now the plasma already contains chlorine ions in higher concentration than is necessary to yield the concentration present in the gastric juice, but the concentration of the hydrogen ions has to be largely increased in the process of secretion, and hence it is evidently upon the concentration of the hydrogen ions in the plasma that the work and speed of separation of hydrochloric acid in the gastric secretion must depend.

Whatever view or theory may be taken of the process; whether the secretion of acid be ascribed to a greater permeability of the oxyntic cell for the hydrogen ion, or a selective absorption for that ion, or an intermediate organic compound be supposed to be formed, or a double decomposition between acid phosphates of alkalies and calcium, or whatever be the supposed process; it is clear that the rate of production of acids, other things being equal, must depend on the concentration of the hydrogen ions in the plasma. A drop from any cause of hydrogen ions in the plasma must mean a corresponding fall in rate of production of acid.

Now blood plasma is a fluid which is, at the same time, alkaline and acid; it contains hydrogen ions and hydroxyl ions, and accordingly affects indicators in different directions (see p. 149). The reaction to phenol-phthalëin shows clearly the presence of hydrogen ions, and the work of the oxyntic cell is to increase the concentration of these ions in the process of secretion.

The concentration of hydrogen ions in the blood plasma is excessively low, so low that it cannot be estimated by such a method as the methyl-acetate inversion method. An attempt has been made by Höber* by the concentration cell method, but the results are so low as to cause one to doubt the accuracy of the method as a quantitative one, although it shows that the amount of effective alkalinity or acidity of the plasma is very low indeed.

---

* Pflüger's 'Arch. f. d. ges. Physiol.,' vol. 81, 1900, p. 535, and 'Physikalische Chemie der Zelle und der Gewebe,' Leipzig, Engelmann, 1902, p. 240.

It is accordingly difficult, without some new method of attack, to investigate the effective acidity of the blood, and it is probable that in the end some physiological method alone, some application, for example, of the effect of minute quantities of acid or alkali upon the rate of growth or activities of living cells, will furnish a delicate enough test for such measurements of reaction as are here required.

It is scarcely necessary to repeat after what has been said under the heading of the methyl-acetate method, that we cannot arrive at the degree of acidity or alkalinity of the blood by simple titration in presence of an indicator. But such determinations ought to be made for the purpose of giving some orientation as to the amounts and relative quantities of the acid and alkali producing salts, the carbonates and phosphates, present in the plasma. A series of such determinations is at present being carried out.

We are hence at present without a method delicate enough to show us how the concentration of the hydrogen ions is varying in the blood plasma in health, or in conditions such as malignant disease, but if we suppose that the failure or reduction in quantity of the acid, is an indication *through the mechanism of the oxyntic cell*, that the concentration of the hydrogen ions in the blood of carcinomatous patients is decreased, and the concentration of the hydroxyl ions increased, then we have indications, from analogy with the changes which occur in other growing cells under like conditions, that such a change would probably give rise to increased cell growth and division.

Thus, Loeb* has shown that addition of 1 c.c. of deci-normal caustic soda solution to 100 c.c. of sea water, that is an addition of only 0·04 gramme per litre, increased the development and growth of the eggs of the sea urchin at such a rate that one could scarcely believe that the two sets of eggs belonged to the same culture. It is only a trace of additional alkali which causes the increased growth, more than a trace stops it entirely.

Now, given a potential tendency to atypical cell growth and mitosis, to reversion to the sexual type of cell-reproduction, it is possible that an increased concentration of hydroxyl ions and diminished concentration of hydrogen ions, would form just the necessary chemical stimulus to start a new growth and determine its continuance and exuberance when started.

It might be urged that the testing of such a view was exceedingly simple, merely by the administration of acid, so as to give hydrogen ions to the blood plasma; but the matter is not so simple as it at first sight appears. It certainly is indicated that some attempt should be made to modify the reaction of the blood plasma, and if possible, restore the acid-secreting function of the oxyntic cells, and we are at present making attempts in this direction.

---

* 'Archiv f. Entwickelungsmechanik,' vol. 7, 1898, p. 631. Quoted from Höber 'Physikalische Chemie d. Zelle u. Gewebe,' p. 235.

But to keep permanently altered, even by continued therapeutic action, the reaction of the blood plasma is by no means easy of attainment. The reaction of the blood is determined by the agency of the liver and kidney cells, and if these have become set at a definite wrong level of action, all the regulating mechanism of the body is then at work against change of the reaction by therapeutic means. When acid is administered, urea is taken and broken up in the body, and ammonia obtained, which is used to neutralise the administered acid.

Hence, it is only with large and continued doses of acid, and on approaching the limits of acid intoxication, that any diminution in alkalinity can be hoped for, and as soon as acid administration is slackened, the acid is neutralised by more ammonia obtained from oxidised proteid.

Thus, even admitting that the diminished acid secretion is due to diminished concentration of hydrogen ions in the blood plasma, we are still face to face with the problem of how to maintain that hydrogen ion concentration in the blood plasma permanently at a higher level, against the competition of the kidney and other cells in the body which are all the time tending to reduce it to its old vicious level.

On Reciprocal Innervation of Antagonistic Muscles.—Seventh Note.

By C. S. Sherrington, F.R.S.

Physiology Laboratory, University of Liverpool.

(Received February 28,—Read April 6, 1905.)

In a previous note* on this subject, instances were pointed out in which the passive posture already obtaining in a limb influences the character of the spinal reflex elicitable from the limb. It was there shown how passive flexion, for instance, favours reflex extension. On resorting to postures assumed and maintained not passively but actively a like influence is evident.

A reflex described in the previous note gives good illustration of this. Light pressure applied to the *planta* of the spinal dog evokes a brisk extension of the limb at hip, knee, and ankle. The pressure applied is such as resembles that which the weight of the dog would, in its own step, apply to the *planta* on contact with the ground. This reflex—which may be termed the "extensor thrust"—employs the antagonist muscles to those employed by the well-known flexion reflex provocable by noxious stimuli applied to the *planta*, *e.g.*, by a prick. This latter reflex, the flexion reflex, although it throws into action a group of muscles different from that thrown into action by the extensor thrust, yet exerts a marked influence over that reaction. If the extensor thrust be compared as elicited before and after a prolonged flexion reflex, the "thrust" reflex is found more facile and vigorous just after the flexion than it was before it.

Again, if the crossed extension reflex of the limb be examined before and after a prolonged flexion reflex a similar alteration is evident in it. When a carefully adjusted electrical stimulus is at regular intervals applied to the afferent path of one limb and the resultant extensor reflex of the crossed limb is noted, it is found that if in one of the intervals a flexion reflex of the latter limb is induced and maintained for 15 seconds or more, the extensor reflex becomes altered in consequence. For a period immediately following the flexion reflex the extension reflex is increased. The intensity of the reflex is heightened its duration is prolonged, and its latent time is reduced. If the testing stimulus be subliminal the threshold value of the stimulus required by the reflex is found lowered. In short the activity of the flexion arcs directly or indirectly induces in the extension arcs a super-excitability as tested by crossed extension just as when tested by the extensor thrust.

---

* 'Roy. Soc. Proc.,' vol. 66, p. 67, 1900.

But although this after-effect of the activity of the flexion arcs upon the antagonistic arcs, both direct and crossed, is one of increase of activity, the primary effect is, as shown previously, one of depression. Thus the crossed extension reflex is temporarily inhibited by the flexion reflex; the extensor thrust becomes inelicitable directly a pronounced flexion reflex sets in. Indeed, these mutually antagonistic reflexes are, like other similarly opposed ones, coupled together in such a way that provocation of the active state of discharge of one reflex checks the active state of discharge of the other reflex. It, therefore, becomes obvious from this, and other evidence given in the previous notes, that in such instances the spinal mechanism, temporarily depressed (inhibited), later enters into a state of exaltation manifested by enhanced tendency to active discharge, and by capacity to discharge with greater intensity than before. A like sequence is well seen when, using the knee jerk as index, the effect on the knee-extensors of stimulation (mechanical, kneading, etc.) of the antagonistic hamstring muscles freed from their attachments is observed. The depression (inhibition) of the antagonists is pronounced, but on discontinuing the stimulation the knee jerk regains not merely its previous briskness, but becomes temporarily more brisk and ample than before. The same is seen when the stimulation is direct faradisation of the afferent nerves from the hamstrings, or when, instead of using the "knee jerk" as an index to the *tonus*, the heightened reflex rigidity of the extensors in decerebrate rigidity is directly observed. In all these instances, and in others that can be given in a fuller communication than the present, there supervenes on the spinal inhibition a rebound effect of augmentation.* This intraspinal rebound effect becomes especially evident on cessation of a stimulus of inhibitory character of *prolonged* application. The rebound can be experimentally shown to ensue even during the actual application of the stimulus that initially caused inhibition if the application of that be long continued. The exaltation after-effect may ensue with such intensity that simple discontinuance of the stimulus maintaining the one reflex is immediately followed by "spontaneous" appearance of the antagonistic reflex. These phenomena are well shown by the opposed reflexes at the knee of the dog.

Thus, in the spinal arcs inhibited there supervenes on the state of inhibition a phase of super-excitability. In this after-effect central inhibition presents resemblance to peripheral inhibition as exemplified, for instance by pure vagus action on the heart. The cardiac depression is followed by exaltation of excitability and conductivity of the cardiac tissue. "As to contraction force and conductivity, the after-effect is in the opposite direction to the primary effect."*

---

* Sherrington, Schäfer's 'Text-book of Physiology,' vol. 2, p. 841, 1900.

This spinal interaction between certain reflex centres, related to movements of opposed direction, resembles that known to hold between adjacent retinal points. The spinal phenomenon seems fundamentally akin to that of visual contrast,† as both Macdougall and myself have pointed out. If visual brightness be regarded as analogous to the activity of spinal discharge, and visual darkness analogous to the absence of spinal discharge, the reciprocal spinal action in the example last mentioned above has a close counterpart in the well-known experiment where a white disc used as a prolonged stimulus leaves as after-effect in the visual field a grey image surrounded by a bright ring (Hering's "Lichthof"). The bright ring has for its spinal equivalent the "spontaneous" discharge from the adjacent reciprocally correlated spinal "centre."‡

The "spinal induction" is obviously qualified to play a part in linking reflexes together in a co-ordinate sequence of successive combination. If a reflex arc A during its own activity not only temporarily checks the discharge-action of an opposed reflex arc B, but also as a subsequent result induces in arc B a phase of greater excitability and capacity for discharge, it predisposes the spinal organ for a second reflex opposite in character to its own in immediate succession to itself. I have previously pointed out the peculiar prominence of "alternating reflexes"§ in prolonged spinal reactions. These may be traceable largely to "spinal induction." It is significant that they are usually cut short with ease by mere passive mechanical interruption of the alternating movement in progress.

Much of the reflex action of the limb that can be studied in the "spinal" dog bears the character of adaptation to locomotion. This has been shown recently with particular clearness by the observations of Philippson.‖ In describing the "extensor thrust" of the limb, I drew attention to its significance for locomotion. "Spinal induction" obviously tends to connect this "extensor thrust" as an after-effect with precurrent flexion of the limb. In the stepping forward of the limb the flexion that raises the foot and carries it forward clear of the ground though temporarily checking the reflex discharge of the antagonistic arcs of extension is, as it continues, so to say, sensitising them to respond later in their turn by the supporting and propulsive extension of the limb necessary to progression. In reflex sequences an antecedent reflex would thus not only be the means of bringing about an ensuing stimulus for the next reflex,* but in such instances as the above, will predispose the arc of the next reflex to react to the stimulus that will arrive.

---

* Gaskell, 'Schäfer's Text-book of Physiology,' vol. 2, p. 220, 1900; see also 'Transact. of VIIIth Internat. Med. Congress,' Copenhagen, 1884.

† Sherrington, 'Journal of Physiol.,' vol. 21, p. 33, 1897; Macdougall, 'Brain,' vol. 26, p. 177, 1903.

‡ For the influence of varying conditions on this experiment and an explanation offered important to the analogy suggested here, see W. Macdougall, "Young's Theory of Light and Colour Vision," 'Mind,' vol. 10, N.S., No. 37, pp. 25 to 30 of the reprint.

§ Croonian Lecture, 1897; 'Phil. Trans.,' B, 1898.

‖ 'Archives de Physiologie,' 1904.

In recently† attempting to deal with the factors that determine the succession of reflexes in time, I mentioned this factor, "spinal induction," but laid less stress on its potency than its phenomena now seem to me really to warrant.

---

* Loeb's "Ketten-reflexe," discussed in his 'Vergleichende Gehirnphysiologie u. Vergleichende Psychologie,' Leipzig, 1899, p. 96, and *seq.;* compare also Exner, 'Entwurf einer physiologischen Erklärung psychischer Erscheinungen,' Vienna, 1894, p. 102, and *seq.*, under "Successive Bewegungscombinationen"; also Wundt, 'Grundzüge der physiologischen Psychologie,' vol. 1, p. 181.

† Brit. Assoc., Cambridge, 1904. Address to Section I.

A Preliminary Note upon the Question of the Nutrition of the Early Embryo, with Special Reference to the Guinea-pig and Man.

By E. Emrys-Roberts, M.B. (Liverpool), Ethel Boyce Research Fellow in Gynæcological Pathology in The University of Liverpool.

(Communicated by Professor C. S. Sherrington, F.R.S. Received February 20,—Read March 16, 1905.)

During the progress of a research into the earliest implantation of the embryo of the guinea-pig, I have been particularly struck with the way in which the nutrition of the embryo is anticipated and provided for during the time it remains free in the uterine horn. The so-called yolk-granules of the ovum are obviously insufficient to provide for the growth of the embryo to the stage prior to differentiation of the inner cell-mass, to which it attains during the five or six days which elapse before it comes into contact with the maternal tissues.* It is clear that it must derive nourishment from the medium in which it lies—the product of the secretion of the uterine or other glands, which, during the period of pro-œstrum, exhibit such marked activity. I suggest that this secretion, which consists of mucus and probably albumin, is assimilated by the embryo after having undergone a process of digestion, the result of a secretory activity on the part of the outermost cells of the embryo—the cells of the Trophoblast. This suggestion I base on my observations in the guinea-pig, where I am able to demonstrate a breaking-down of maternal cells before the Trophoblastic cells are in actual contact; likewise in human placentation where a more or less dense layer of fibrin and broken-down leucocytes and decidual cells, the result of Trophoblastic activity, affords a barrier interposed between the invading Trophoblastic cells and the Decidua. This layer I purpose naming the "Protective Layer."

Looked at from a comparative point of view, there is in all probability a close analogy between the uterine secretion of mammals, and the secretion of the oviducts of the lower vertebrata. In the case of birds the analogy is very striking, on account of the direct and important share in the nutrition of the embryo afforded by this secretion, commonly known as the white of the egg. In the case of the frog the ovum receives in its passage down the oviduct, corresponding to the uterine horn of the guinea-pig, a coating of mucus and probably albumin, comparable to the uterine secretion referred to above; when it reaches the water and becomes fertilised, this swells up by absorption, forming a gelatinous covering. The embryo for nutriment depends upon the yolk contained in the ovum before fertilisation, upon the covering of mucus and probably albumin, and lastly upon the water in which it lies. In certain mammals, as, for example, the rabbit and the mole, a distinct gelatinous envelope is described as surrounding the embryo before implantation occurs; this envelope is, I suggest, possibly due to some digestive action of the cells of the Trophoblast upon the adjacent medium, producing a form of coagulation.

---

* This insufficiency is even more pronounced in the mole, where the uterine cavity is actually distended by the growing embryo before implantation takes place.

In a number of mammals during the period of pro-œstrum the secretion of uterine glands is supplemented by a pouring-forth of blood, from the simple oozing of congested vessels, to that associated with actual exfoliation of uterine epithelium. Although there is reason to suppose that the flow of blood has practically ceased before the embryo reaches the uterine cavity, there is, in all probability, a considerable quantity of blood-serum present, in addition to the secretion of the uterine glands. I think it is conceivable, if not highly probable, that this process is, in part at least, a preparation on the part of the mother for providing a rich pabulum to nourish the embryo until such time as it attaches itself to the uterine wall; while, when implantation is effected, there is in readiness for the embryo an abundant supply of richest nutriment.

The Influence of Cobra-venom on the Proteid Metabolism.

By JAMES SCOTT, M.D., C.M., B.Sc. Edin.

(Communicated by Sir Thomas R. Fraser, F.R.S. Received February 6,—
Read April 6, 1905.)

(From the Research Laboratory of the Royal College of Physicians, Edinburgh.)

Although so much valuable work has been done upon the physiological action of Cobra-venom by Fraser, Calmette, Elliot and others,* so far no observations of its effects upon the metabolism have been recorded. While its peculiarly selective action on the central nervous system would seem to suggest the absence of any marked general action, the demonstration afforded by Elliot's work of its influence and the way in which peripheral nerve mechanism may be attacked, and of its interference with respiration, and the direct local action on the tissues into which it is injected, seem to indicate that its toxic action may extend to protoplasm generally and that it may thus lead to modification of the proteid metabolism, whether in the direction of altering the rate of proteid metabolism or of modifying the synthetic processes in the liver by which urea is elaborated, as do the toxins of certain micro-organisms.

I have to thank Sir Thomas R. Fraser for his kindness in giving me the Cobra-venom; and also to thank Dr. Noël Paton for much valuable assistance in this investigation.

General Plan of Investigation and Methods.

Dogs were used for this investigation. Before each experiment they were fed for some days on a fixed diet of oatmeal porridge and milk in order to establish nitrogenous equilibrium. The urine was collected by keeping the animal in a cage with a sloping bottom made of zinc under which a suitable vessel was placed. The floor was kept scrupulously clean, and fæces were removed as soon as possible after they were passed. The urine was collected daily at 10 A.M.

When the animal came into nitrogenous equilibrium, sub-lethal and in some cases lethal doses of Cobra-venom were injected subcutaneously.

Urine.—The reaction and specific gravity were taken. The quantity collected was noted and the urine diluted to a convenient volume. The amounts of the following ingredients were determined by the methods enumerated, duplicate analyses were made in all cases and the mean of these taken:—

---

* See Fayrer: 'Thanatophidia of India'; Brunton and Fayrer, 'Roy. Soc. Proc.,' vol. 22, 1874; Wall, 'Indian Snake Poisons,' 1883; Nicholson, 'Ophiology,' 1893.

1. Total nitrogen by Argutinsky's modification of Kjeldahl's method.
2. Nitrogen in urea by Bohland's method by precipitating with phosphotungstic (Merck's) and hydrochloric acids.
3. Nitrogen of ammonia by Schlössing's method.
4. Nitrogen not in urea (non-urea nitrogen) was calculated by difference.
5. Nitrogen in purin bodies by Krüger and Wulff's method.*
6. Phosphoric acid as P_2O_5 by titrating with uranium nitrate.
7. The percentages of nitrogen in urea, not in urea (non-urea nitrogen), in ammonia, in purin bodies, in other compounds were calculated in terms of total nitrogen.
8. The percentages of P_2O_5 were calculated in terms of total nitrogen.

Averages of the figures before and after the injections were determined.

Albumin was tested for by cold nitric acid and by heat and acetic acid.

Some of the experiments were rendered valueless by the early development of an abscess at the seat of inoculation, but the following were carried on, without disturbance from such accidents.

In all the experiments no marked symptoms were produced. In some cases after injection the dog lay very quiet for a few hours. In a few there was a local swelling observed next day, but it soon disappeared.

Experiment 1.

For this experiment a retriever weighing 18 kilogrammes was used. The dog was kept in its cage for two or three days before the examination of the urine was begun, so that it might get accustomed to its diet and nitrogenous equilibrium be established.

Food Analyses.

Analyses of the food given to the dog showed the amount of nitrogen to be:—

Daily diet:	500 c.c. milk.....................	= 1·83 grammes nitrogen
	200 grammes oatmeal	= 4·30 „
	Total intake of nitrogen per diem...	= 6·13 „

Analyses of the urine were begun on November 16, 1903, and on the 19th, at 4 P.M., 1·25 milligrammes Cobra-venom dissolved in 0·5 c.c. sterilised normal saline solution was injected subcutaneously in the side.

---

* *Cf.* 'Zeit. für Phys. Chem.,' vol. 20, p. 177, 1895.

Table

Date.	Quantity of urine in c.c.	Sp. gr.	Re-action.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.
1903—									
November 16 ...	380 diluted to 500	1023	acid	1·098	5·45	0·882	4·11	0·216	1·36
„ 17 ...	315 to 500	1018	acid	0·734	3·67	0·566	2·83	0·168	0·84
„ 18 ...	380 to 500	1020	acid	0·927	4·63	0·759	3·79	0·168	0·84
							1·25 milligrammes Cobra		
November 19	500	1021	acid	1·210	6·05	0·959	4·79	0·251	1·26
„ 20	500	1015	acid	0·820	4·102	0·622	3·11	0·198	0·99
„ 21 ...	385 to 500	1019	acid	0·811	4·053	0·603	3·01	0·208	1·04
„ 22 ...	435 to 500	1021	acid	0·977	4·89	0·829	4·14	0·148	0·75
„ 23	500	1021	acid	1·064	5·32	0·874	4·37	0·190	0·95
„ 24 ...	430 to 500	1015	acid	0·692	3·458	0·573	2·86	0·176	0·88
							2·50 milligrammes Cobra		
November 25	500	1015	acid	0·778	3·892	0·634	3·17	0·144	0·72
„ 26	600	1015	acid	0·746	4·477	0·547	3·29	0·197	0·98
„ 27	700	1015	acid	0·697	4·609	0·566	3·96	0·131	0·64
„ 28	500	1015	acid	0·784	3·920	0·591	2·95	0·193	0·97
„ 29 ...	250 to 500	1016	slightly alkaline	0·3738	1·869	0·2702	1·35	0·1036	0·51
„ 30	600	1015	acid	0·7098	4·259	0·585	3·51	0·1286	0·74
							Averages Before and		
November 16, 17, and 18 (before injection)				0·919	4·59	0·736	3·58	0·184	1·01
„ 19, 20, 21, and 22 (after 1st injection).				0·954	4·77	0·753	3·76	0·201	1·01
„ 25, 26, 27 and 28 („ 2nd „).				0·751	4·22	0·585	3·34	0·166	0·83

I.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
0·0728	0·364	0·0078	0·0390	0·281	1·405	80	20	6·6	0·71	12·69	26
0·0301	0·105	0·0097	0·0485	0·147	0·735	77	23	4·1	1·32	17·58	20
0·056	0·280	0·0047	0·0238	0·221	1·105	82	18	6·04	0·51	11·45	24
venom injected at 4 P.M.											
0·0889	0·4445	0·0092	0·0462	0·377	1·885	88	12	7·34	0·76	3·90	31
0·0497	0·2485	0·0098	0·0490	0·213	1·065	76	24	6·06	1·20	16·74	26
0·0596	0·2982	0·0154	0·0770	0·232	1·160	74	26	7·35	1·90	16·75	28·6
0·0902	0·4508	0·0084	0·0420	0·299	1·495	85	15	9·22	0·86	4·92	30·6
0·0714	0·3570	0·0087	0·0434	0·357	1·785	82	18	6·71	0·82	10·45	33
0·0560	0·2800	0·0073	0·0364	0·191	0·955	83	17	8·23	1·05	7·72	27·6
venom injected at 9.30 A.M.											
0·0612	0·3059	0·0094	0·0469	0·181	0·905	82	18	8·0	1·20	8·80	23·3
0·0938	0·5628	0·0070	0·0420	0·227	1·362	75	25	12·4	0·94	11·66	30·4
0·1008	0·7056	0·0077	0·0539	0·235	1·645	81	19	14·4	1·15	3·45	33·7
0·0916	0·4578	0·0104	0·0518	0·203	1·015	75	25	11·7	1·34	11·96	26
0·0885	0·4424	0·0062	0·0308	0·076	0·380	72	28	10·4	1·45	16·15	18
0·0641	0·3847	0·0073	0·0437	0·223	1·338	82	18	9·03	1·02	7·95	31
After the Injection.											
0·053	0·249	0·0074	0·0371	0·216	1·08	80	20	5·58	0·85	13·91	23
0·072	0·360	0·0107	0·0586	0·280	1·40	80	20	7·99	1·18	10·58	29
0·062	0·508	0·0084	0·0511	0·236	1·23	78	22	11·6	1·16	8·97	28

Table II.—Weight

Date.	Quantity of urine in c.c.	Sp. gr.	Re-action.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.
1904—									
March 22.........	750 diluted to 1000	1015	acid	0 ·552	5 ·52	0 ·455	4 ·55	0 ·097	0 ·97
„ 23.........	710 to 1000	1014	acid	0 ·504	5 ·04	0 ·434	4 ·34	0 ·070	0 ·70
„ 24.........	550 to 1000	1020	acid	0 ·585	5 ·85	0 ·490	4 ·90	0 ·095	0 ·95
„ 25.........	550 to 1000	1019	acid	0 ·543	5 ·43	0 ·465	4 ·65	0 ·078	0 ·78
							5 milligrammes Cobra		
March 26.........	265 to 500	dark brown 1027	strongly acid	0 ·714	3 ·57	0 ·579	2 ·89	0 ·135	0 ·675
„ 27.........	610 to 1000	yellow 1013	acid	0 ·403	4 ·03	0 ·322	3 ·22	0 ·081	0 ·81
„ 28............	1000	1015	acid	0 ·799	7 ·99	0 ·643	6 ·43	0 ·156	1 ·56
„ 29.........	650 to 1000	1015	acid	0 ·484	4 ·48	0 ·389	3 ·89	0 ·095	0 ·95
„ 30.........	800 to 1000	1016	acid	0 ·711	7 ·11	0 ·601	6 ·01	0 ·110	1 ·10
							Averages Before and		
March 22 to 25 (inclusive) (before injection)				0 ·546	5 ·46	0 ·461	4 ·61	0 ·085	0 ·85
„ 26 to 29 („) (after „)				0 ·600	5 ·11	0 ·483	4 ·11	0 ·117	0 ·99

of Dog, 17 Kilogrammes.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
0 ·0386	0 ·386	0 ·0059	0 ·059	0 ·185	1 ·85	82	18	7 ·0	1 ·06	9 ·94	33
0 ·0311	0 ·311	0 ·0055	0 ·055	0 ·158	1 ·58	86	14	6 ·2	1 ·08	6 ·72	31
0 ·0330	0 ·330	0 ·0069	0 ·069	0 ·187	1 ·87	84	16	5 ·6	1 ·17	9 ·23	32
0 ·0361	0 ·361	0 ·0064	0 ·064	0 ·154	1 ·54	86	14	6 ·7	1 ·18	6 ·12	28
venom injected at 11 A.M.											
0 ·0434	0 ·217	0 ·0053	0 ·0265	0 ·237	1 ·185	81	19	6 ·1	0 ·75	12 ·15	33
0 ·0325	0 ·325	0 ·0130	0 ·130	0 ·130	1 ·30	80	20	8 ·1	3 ·23	8 ·67	32
0 ·0462	0 ·462	0 ·0087	0 ·087	0 ·234	2 ·34	80	20	5 ·8	1 ·09	13 ·11	29
0 ·0325	0 ·325	0 ·0073	0 ·073	0 ·141	1 ·41	81	19	6 ·7	1 ·50	10 ·8	29
0 ·0448	0 ·448	0 ·0102	0 ·102	0 ·180	1 ·80	85	15	6 ·3	1 ·44	7 ·26	25
After the Injection.											
0 ·0347	0 ·347	0 ·0062	0 ·062	0 ·171	1 ·71	84	16	6 ·4	1 ·12	8 ·0	31
0 ·0386	0 ·332	0 ·0086	0 ·079	0 ·185	1 ·56	81	19	6 ·7	1 ·64	11 ·18	31

Table

Date.	Quantity of urine in c.c.	Sp. gr.	Re-action.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.
						20 milligrammes Cobra venom (two minimum			
1904— April 8	No urine	passed.							
" 9	760 to 1000	1015	acid	0·603	6·03	0·518	5·18	0·085	0·85
" 10	690 to 1000	1015	acid	0·483	4·83	0·409	4·09	0·074	0·74
" 11	500 to 1000	1015	acid	0·440	4·40	0·372	3·72	0·068	0·68
								Averages after	
April 9, 10, and 11				0·509	5·09	0·433	4·33	0·076	0·76

The minimum lethal dose of the venom as determined is 0·00025 gramme per kilogramme of body weight. Aseptic precautions were used. The effects of this injection were studied for five days and a second injection of 2·5 milligrammes Cobra venom dissolved in 1 c.c. saline solution was injected on the 25th at 9.30 A.M. and the examination of the urine carried on till November 30.

Experiment 2.

For this experiment a collie, weighing 17 kilogrammes, was used. The same precautions were taken and the same amount of food given.

The analyses of the urine were begun on March 22, 1904, and on the 26th 5 milligrammes Cobra venom in 0·5 c.c. sterilised normal saline solution were injected subcutaneously in the side at 11 A.M. A few hours after the injection the dog vomited, but there was no other symptom. The effects of the venom were studied till the 30th.

III.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other compounds.	P_2O_5.
lethal doses) injected at 1 P.M. on April 8.											
0·0408	0·408	0·0084	0·084	0·104	1·04	86	14	6·8	1·39	5·81	17
0·0314	0·314	0·0076	0·076	0·111	1·11	85	15	6·5	1·57	6·93	23
0·0316	0·316	0·0066	0·066	0·124	1·24	85	15	7·2	1·50	6·30	28
the injection.											
0·0346	0·346	0·0075	0·075	0·113	1·13	85	15	6·8	1·49	6·35	23

Experiment 3.

The same collie was used for this experiment. On April 8, at 1 P.M., 20 milligrammes Cobra venom in 2 c.c. sterilised normal saline solution were injected subcutaneously. This amount is equivalent to two minimum lethal doses. No urine was passed on the 8th. The urine of the 9th, 10th, and 11th was examined. On the 11th an abscess was observed below the seat of inoculation and the animal was destroyed by chloroform.

Experiment 4.

For this experiment a retriever, weighing 12 kilogrammes, was used. The analyses were begun on June 30 and were continued till July 12. On the 6th, 7th, and 8th July 3·3 milligrammes Cobra venom were injected subcutaneously each day at 3 P.M.

Table IV.—Weight

Date.	Quantity of urine in c.c.	Sp. gr.	Re-action.	Nitrogen.					
				Total nitrogen.		Nitrogen as urea.		Nitrogen not as urea.	
				Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.
1904—									
June 30	600 diluted to 1000	1012	acid	0·369	3·69	0·311	3·11	0·058	0·58
July 1	350 to 500	1016	acid	0·515	2·576	0·423	2·11	0·092	0·46
„ 2	600 to 1000	1013	acid	0·386	3·86	0·322	3·22	0·064	0·64
„ 3	440 to 500	1015	acid	0·605	3·02	0·526	2·63	0·079	0·39
„ 4	600 to 1000	1010	acid	0·274	2·74	0·249	2·49	0·025	0·25
„ 5	500	1015	acid	0·658	3·29	0·526	2·63	0·132	0·66
„ 6*	800 to 1000	1010	acid	0·288	2·88	0·218	2·18	0·070	0·70
„ 7*	270 to 500	1030	acid	0·470	2·35	0·342	1·81	0·128	0·54
„ 8*	100 to 500	1048	acid	0·595	2·97	0·479	2·39	0·116	0·58
„ 9	200 to 500	1030	acid	0·616	3·08	0·489	2·44	0·127	0·64
„ 10	500	1013	acid	0·552	2·76	0·426	2·13	0·126	0·63
„ 11	500	1013	acid	0·655	3·27	0·496	2·48	0·159	0·795
„ 12	600	1012	acid	0·535	3·11	0·445	2·67	0·090	0·538
				Averages of six days before					
June 30, July 1, 2, 3, 4, and 5 (before injections).				0·468	3·197	0·393	2·52	0·075	0·49
July 6, 7, 8, 9, 10, and 11 ......(after „).				0·529	2·88	0·42	2·24	0·121	0·65

* On July 6, 7, and 8, Cobra venom 3·3 milligrammes injected each day.

of Dog, 12 Kilogrammes.

Nitrogen in ammonia.		Nitrogen in purin bodies.		Phosphorus as P_2O_5.		Percentages of—					
Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.	Per-centage of urine.	Per diem.	Nitrogen as urea.	Nitrogen not as urea.	Nitrogen in ammonia.	Nitrogen in purin bodies.	Nitrogen in other com-pounds.	P_2O_5.
0·0244	0·244	0·0042	0·042	0·132	1·32	84	16	6·6	1·14	8·26	33
0·0468	0·234	0·0062	0·031	0·201	1·005	82	18	9·0	1·20	9·80	39
0·0417	0·417	0·0052	0·052	0·162	1·62	83	17	10·8	1·34	4·46	42
0·0582	0·291	0·0070	0·035	0·180	0·90	87	13	9·6	1·16	2·24	30
0·0238	0·238	0·0050	0·050	0·106	1·06	90	10	8·7	1·84	0·0	39
0·0560	0·280	0·0099	0·0497	0·280	1·40	80	20	8·5	1·43	10·07	43
0·0375	0·375	0·0039	0·039	0·120	1·20	76	24	13·0	1·36	9·64	42
0·0375	0·1875	0·0045	0·0225	0·206	1·03	73	27	8·0	1·04	17·96	44
0·0465	0·2325	0·00728	0·0364	0·250	1·25	80	20	7·8	1·22	10·98	42
0·0538	0·2690	0·00938	0·0469	0·270	1·35	79	21	8·7	1·52	10·78	44
0·0566	0·2828	0·01288	0·0644	0·228	1·14	77	23	12·5	2·33	8·17	41
0·0582	0·2910	0·01256	0·0628	0·243	1·21	76	24	8·9	1·92	13·8	37
0·0557	0·3343	0·01008	0·0604	0·189	1·13	83	17	14·0	1·90	1·1	35
and six days after the injection.											
0·0418	0·2840	0·00625	0·0433	0·177	1·217	84	16	8·9	1·35	5·8	38
0·0483	0·2730	0·0086	0·0453	0·219	1·19	77	23	9·8	1·56	11·79	42

General Results.

No albumin was ever passed as the results of any injections.

	Experiments.							
	I.		II.		III.		IV.	
	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.	Percentage of urine.	Per diem.
Averages before injection of venom	0·919	4·59	0·546	5·46	0·546	5·46	4·68	3·197
Averages after 1st injection	0·954	4·77	0·600	5·11	—	—	—	—
Averages after 2nd injection	0·751	4·22	—	—	0·509	5·09	—	—
After three injections							5·29	2·88

In no case was there any indication of an increased proteid metabolism.

Distribution of Nitrogen in Urea and Other Compounds.

Percentages of Urea Nitrogen to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection	80	84	84	84
„ after first injection	80	81	—	—
„ „ second injection	78	—	85	—
After three injections				77

In three of the experiments there was a slight fall in the production of urea nitrogen, most marked in Experiment IV where three doses in succession had been given.

Percentages of Ammonia Nitrogen to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection	5·57	6·4	6·4	8·9
„ after first injection	7·99	6·7	—	—
„ „ second injection	11·6	—	6·8	—
After three injections				9·8

In Experiment I there was a marked rise and in Experiment IV a slight rise, while the changes in the other two were in the same direction.

Percentages of Nitrogen in Purin Bodies to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection............	0·85	1·12	1·12	1·35
„ after first injection	1·18	1·64	—	—
„ „ second injection ...	1·16	—	1·49	—
After three injections......				1·56

These changes show a slight rise in each experiment.

Percentages of Nitrogen in Other Compounds to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection............	13·91	8·11	8·11	5·8
„ after first injection	10·58	11·18	—	—
„ „ second injection ...	8·89	—	6·35	—
After three injections......				11·78

In two experiments there was a rise and in two a fall.

Percentages of P_2O_5 to Total Nitrogen.

	Experiments.			
	I.	II.	III.	IV.
Averages before injection	23	31	31	38
„ after first injection.........	29	31	—	—
„ „ second injection ...	28	—	23	—
After three injections......				42

In two experiments a rise, in one no change, and in one a fall. The variations in the P_2O_5 do not correspond to the changes in the purin bodies.

Conclusions.

1. Practically no change in rate of proteid metabolism was induced by the administration, in spite of well marked local reaction.

2. A slight decrease in the proportion of urea nitrogen, quite insignificant compared with that produced by diphtheria toxine and various drugs, was observed.

3. A slight rise in the proportion of ammonia nitrogen occurred.

4. There was a slight rise in the proportion of nitrogen in purin bodies.

5. The nitrogen in other compounds showed no constant change.

6. The P_2O_5 excreted showed no constant change, but in two experiments there was a slight rise.

The change produced in the proteid metabolism is, therefore, small, and such as it is, being in the directions of decreased elaboration of urea and increase in the proportion of nitrogen excreted as ammonia, it seems to indicate a slight toxic action on the hepatic metabolism rather than a general action on the proteid changes; and tends to confirm the view that the poison acts chiefly upon the nervous system.

On the Physical Chemistry of the Toxin-Antitoxin Reaction: with Special Reference to the Neutralisation of Lysin by Antilysin.

By J. A. Craw, Research Student, Lister Institute of Preventive Medicine.

(Communicated by Dr. C. J. Martin, F.R.S. Received March 22,—Read April 6, 1905.)

Ehrlich (1898) (1903) came to the conclusion that the toxin secreted by *B. diphtheriæ* is neutralised by diphtheria antitoxin much as an acid is by a base. The course of the neutralisation seems to him to indicate the presence of several toxic substances and atoxic forms of the same substances in the toxic fluid which are successively neutralised by the gradual addition of antitoxin. After complete neutralisation of the various toxins, a substance—toxone—remains which has the property of causing diphtheritic paralysis, and also of neutralising antitoxin. Similar constitutions have been ascribed by Ehrlich and his pupils to other toxic fluids, and to the hæmolytic filtrates or lysins obtained from certain bacterial cultures.

On the other hand, Arrhenius and Madsen (1902) (1904) concluded that the toxin-antitoxin reaction is quite analogous to the action of an acid on an alcohol, and that the chemical laws of mass action, which hold for the latter, apply equally well to the former. The chief reaction is considered to be a reversible one between two substances only, toxin and antitoxin, and when the system has reached equilibrium, a fraction of the toxin and also of the antitoxin remain free. The toxone effect they ascribe to a trace of free toxin. The numerical relations deduced are approximately in agreement with the experimental observations they have made on equilibria obtaining between toxins and antitoxins, and likewise between lysins and antilysins. Nernst (1904) has, however, pointed out that the laws of mass action are not applicable to these reactions.

Bordet (1903) expressed the view that the fixation of toxin by antitoxin is similar to the fixation of a dye by a tissue, and the author has shown that this conception is consonant with the chemical and physical properties of antibodies in general (1905).

The two substances most thoroughly investigated by Arrhenius and Madsen, viz., diphtheria toxin and tetanus lysin, do not admit of exact determination. The estimation of the free diphtheria toxin is rendered uncertain by reliance on animal experiments, and tetano-lysin is itself a most unstable body. Todd (1902), however, discovered a relatively stable lysin in the filtrate from cultures of *B. megatherium*, and succeeded in producing a very strong antilysin for the same. He demonstrated that, in constitution, this lysin resembled tetano-lysin and diphtheria toxin. I, therefore, determined to test in how far the various views were applicable to the relations existing between Megatherium lysin and antilysin. Dr. G. Dean kindly placed the antilysin prepared by Dr. Todd at my disposal. I here beg to express my deep sense of indebtedness to Dr. G. Dean for his kindly criticism and many suggestions, and to Dr. C. J. Martin for his counsel in the filtration experiments.

On the hæmolytic index employed.—That the hæmolytic effect is always proportional to the amount of lysin added seems to be more than doubtful, consequently the calculated concentrations of free lysin have not been given.

However, when the hæmolytic powers of two solutions of the same lysin in the presence of a considerable concentration of antilysin are not widely different, the powers are approximately proportional to the amounts of free lysin present. The hæmolytic power of a fluid was determined by adding 1 c.c. to 2 c.c. of a 2·5-per-cent. suspension of washed guinea-pig corpuscles in 0·8-per-cent. saline, and heating the mixture to 37° C. for three hours.

The contents were subsequently allowed to stand in the ice-chest until the corpuscles had sedimented sufficiently to allow of 1 c.c. of the supernatant fluid being removed. The intensity of colour of the fluid was then compared with that of the tinted scale of a von Fleischl's hæmoglobinometer. By using a disc of paper stained with potassium chromate, instead of the usual white illumination, the contrast in colour between hæmoglobin and scale is much diminished, and daylight may be used for the comparison. Only that portion of the scale between the numbers 30 and 70 was used, and when the fluid was strongly coloured, dilutions were made so as to bring the final tint within these limits. The scale was standardised by various dilutions of the fluid obtained when 1 c.c. of lysin completely hæmolysed 2 c.c. of 2·5-per-cent. corpuscles in saline. The intensity of tint of this fluid has been represented by the index 100, and the hæmolytic indices given below refer to this tint as a standard. The experimental error in determining the tint was found to be less than 1 per cent. of the standard tint.

In the following a "partially neutralised" mixture of lysin and antilysin is a fluid giving a marked hæmolysis under standard conditions; a "neutral" mixture gives no hæmolysis in three hours, but would hæmolyse slightly in four hours; an "over-neutralised" mixture contains a greater proportion of antilysin than a neutral mixture.

On the presence of Free Lysin and Free Antilysin in mixtures which have attained equilibrium. Method: Filtration through Gelatine.

The method employed was that of C. J. Martin (1896), and applied by Martin and Cherry (1898), in their investigation of the relations existing between diphtheria toxin and antitoxin. The fluids examined were forced by a constant air-pressure of 100 atmospheres through Pasteur-Chamberland filters filled with solid gelatine, and each filtrate was removed in successive fractions of about 4 c.c. The hæmolytic powers of these fractions of filtrates, of the residual fluid left in the filter case, and of the original fluid introduced, were then determined in the manner described above.

Lysin.—On filtering a weak lysin solution through a filter prepared from 15-per-cent. gelatine, the filtrate gave little or no hæmolytic effect, but on diminishing the concentration of gelatine to 9 per cent., and using a stronger lysin, the filtrates obtained were strongly hæmolytic. The first few fractions had practically no action on blood corpuscles, and the succeeding fractions increased at first rapidly in hæmolytic power and then more slowly until a fairly constant value was reached, which approximated to that of the original lysin introduced. The gelatine, also, proved to be intensely hæmolytic, whereas the original gelatine had no effect on red-blood corpuscles. Candles prepared from 15-per-cent., 11-per-cent., 9-per-cent., and 7·5-per-cent. gelatine were also used, and the residual fluids showed in all cases a greater hæmolytic power than the original lysin, the difference being more marked with the higher percentages of gelatine. The gelatine filter is evidently more permeable to water than to the lysin. Similar concentration relations were found to hold for the filtration of crystalloids and inferior colloids, but the filter showed considerable differences in permeability to various crystalloidal substances.*

Antilysin.—On filtering a 5-per-cent. solution of the antilysin in saline no trace of anti-hæmolytic action could be detected in the filtrates. In this respect the antilysin behaves like a typical colloid, *e.g.*, colloidal ferric hydrate was found even in concentrated solutions to be almost entirely retained by the gelatine on filtering under *constant* pressure. It is important to observe that if the pressure be *suddenly diminished* the concentrated contents of the gelatine, whether crystalloidal or colloidal, are swept into the filtrate. The filter showed considerable permeability to typical colloids, especially those which stained the gelatine, but on the whole retained this class of substances to a greater extent than crystalloids.

---

* *Cf.* also E. W. Reid, 1901.

Mixtures.—The mixtures of lysin and antilysin were brought to a state of equilibrium by heating at 37° C. before filtering. They embraced weakly hæmolytic fluids (Nos. 1, 2, and 3, Table I), neutral fluids, *i.e.*, mixtures which did not hæmolyse in the standard time (No. 4), and fluids containing a large excess of antilysin (Nos. 5 and 6).

Nos. 1, 2, and 3 were exactly of the same constitution, viz., equal volumes of a lysin of constant value and a 5-per-cent. solution of antilysin in saline. No. 1 was heated 1 hour at 37° C., and kept at 10° C. for 1 hour. Nos. 2 and 3 were heated for 3 hours at 37° C., and allowed to stand 18 hours at 10° C.*

No. 4 consisted of equal volumes of a 1-per-cent. solution of a lysin which had been precipitated by ammonium sulphate, and of a 5-per-cent. solution of antilysin in saline. No. 5 contained one volume of the 1-per-cent. lysin to two volumes of 5-per-cent. antilysin, and No. 6, one volume of 1-per-cent. lysin to four volumes of 5-per-cent. antilysin. Nos. 4, 5, and 6 were heated for 3 hours at 37° C., cooled 1 hour at 10° C. and filtered.

Examination of the Filtrates for the Presence of Lysin.—The last fractions of the filtrate from No. 1 indicated a trace of hæmolysis, the corresponding fractions from Nos. 2 and 3 were unquestionably hæmolytic.

The filtrates from Nos. 4, 5, and 6, as well as the original fluids introduced, did not produce hæmolysis.

Examination of the Gelatine for the Presence of Lysin.—After filtration the *gelatine* of the filters 1 to 6 was melted out at 37° C., and *in all cases was found to be intensely hæmolytic,* whereas the original gelatine had no hæmolytic effect in the standard time.

Examination of the Residual Fluids for the Presence of Lysin.—The residual fluids were in all cases decidedly hæmolytic, as can be seen in Table I. *This increment in hæmolytic power was to be expected for Nos.* 1, 2, *and* 3 *from the results given above for the filtration of lysin alone,* and is simply a concentration effect that might be brought about by removing the water in other ways, *e.g.*, by evaporation under diminished pressure. The result, however, appears to be most remarkable when it is considered that No. 4 is a *neutral mixture*, and Nos. 5 and 6 *highly over-neutralised.*

Control filtrations of saline showed no hæmolytic power in either filtrate, residue, original fluid, or gelatine, so that the hæmolysis obtained above was certainly not due to impurities introduced by the apparatus.

---

* The experiment with No. 3 was performed 30 days after the experiments with Nos. 1 and 2 and the agreement in the hæmolytic values obtained testifies to the constancy of the lysin.

Table I.

Fluids mixed	Fluid lysin KXII. Antilysin 5 per cent. in saline.						Precipitated lysin 1 per cent. in saline. Antilysin 5 per cent. in saline.							
No. of experiment	1		2		3		4		4A		5		6	
Volume ratio of lysin to antilysin	1 : 1		1 : 1		1 : 1		1 : 1		—		1 : 2		1 : 4	
Temperature and time of contact	37° 1 hr. 10° 1 hr.		37° 3 hrs. 10° 18 hrs.		37° 3 hrs. 12° 18 hrs.		37° 3 hrs. 10° 1 hr.		37° 2½ hrs. 10° 1 hr.		37° 3 hrs. 10° 1 hr.		37° 3 hrs. 10° 1 hr.	
	Vol. in c.c.	Hæm. index.	Vol. in c.c.	Hæm. index.	Vol. in c.c.	Hæm. index.	Vol. in c.c.	Hæm. index.	Vol. in c.c.	Hæm. index.	Vol. in c.c.	Hæm. index.	Vol. in c.c.	Hæm. index.
Last fraction of filtrate	5	1·0	4	7·7	16	4·5	4	0	4	0	4	0	4	0
Total filtrate	75	0·5	63	6·3	72	2·8	54	0	62	0	48	0	35	0
Residue	40	38·6	35	41·3	37	38·2	33	31·2	33	27·8	42	21·6	15	20·1
Gelatine	—	100	—	100	—	100	—	100	—	100	—	100	—	100
Original mixture	120	16·8	105	24·3	112	16·8	98	0	100	0	100	0	62·5	0

The hæmolytic power of the residues and the gelatine could be demonstrated to be due to free megatherium lysin, for on the addition of sufficient antilysin *the hæmolytic power was in all cases entirely neutralised.* For example, 1 c.c. of residue No. 1 of index (38·6) with 1 c.c. of 5-per-cent. antilysin, added directly to the test blood, gave a hæmolytic index of (8·4), the same mixture heated for one hour at 37° C. before adding to the test blood, gave as index (1·8). Again, 0·5 c.c. of gelatine from No. 1 caused complete hæmolysis (index 100), whereas, after being heated for 1 hour at 37° C. with 1 c.c. of 5-per-cent. antilysin the value was reduced to (25). Free lysin, then, exists in the residues, and since the filtration was completed in less than two hours at a temperature of 10° C., at which temperature the velocity of reaction is extremely low, there can have been no appreciable liberation or dissociation of lysin during the filtration. It follows, therefore, that *free lysin exists in neutral and highly over-neutralised mixtures of lysin with antilysin, and that the free lysin is partially removed during filtration.*

The Existence of Free Antilysin in Partially neutralised and Neutral Mixtures.

The residual fluids from Experiments 1 to 6 showed slightly smaller hæmolytic indices after standing over night at 10° C., and on heating them to 37° C. for 1 hour their hæmolytic powers further markedly decreased. This is clearly shown by the following experiment:—

1 c.c. of residue No. 1 (index 38·6) together with 1 c.c. of saline, when added directly to the test blood, gave a hæmolytic index of (35), whereas the same mixture heated for one hour at 37° C. before being added to the test blood, gave an index of only (23·4). This behaviour indicates that in Nos. 5 and 6, the filtration which causes an increase in the concentration of any free lysin or antilysin by the withdrawal of water, occasions a further reaction which has a low velocity at 10° C., but is considerably more rapid at 37° C. As this phenomenon is common to all the residues it follows that if the above interpretation be correct, free antilysin is present in all cases, and presumably must have been present in the original mixtures. Nos. 1, 2, and 3 were, however, hæmolytic mixtures, and No. 4 neutral, which points to the conclusion that *free antilysin exists in partially neutralised and neutral mixtures* as well as in over-neutralised mixtures.

This conclusion is strengthened by the high neutralising power possessed by the original mixture and by the residue of No. 3, when fresh lysin was added, as shown by the following experiments:—

To one series of tubes containing 1 c.c. of the original hæmolytic mixture in equilibrium, gradually increasing quantities of lysin were added. The same quantities of lysin were added to a second series containing 1 c.c. of the residue of No. 3, and to a third series containing 1 c.c. of saline. The test blood was added to each and, after mixing, the tubes were placed at 37° C. for 3 hours. The hæmolytic indices obtained are shown in Table II.

Table II.

Lysin K_{XII} added, in c.c.	Hæmolytic indices.				
	Saline.	Original mixture, No. 3.	Residual fluid, No. 3.	Original mixture increments.	Residual fluid increments.
0	0	25	48·2	—	—
0·0001	0	25	48·2	0	0
0·001	0	25	48·2	0	0
0·01	2·3	25	48·2	0	0
0·1	100	33·2	55·0	8·2	6·8
0·2	100	41·4	59·1	16·4	10·9
0·3	100	50·9	63·2	25·9	15·0
0·4	100	59·1	67·2	34·1	19·0

Obviously the original mixture has neutralised a very considerable proportion of the added lysin, for 0·1 c.c. of the lysin causes complete hæmolysis in saline (index 100), whereas 0·3 c.c. in the mixture only produces half the effect (index 50·9). The residue, although itself more hæmolytic than the original mixture, shows a higher neutralising value than the latter, as may be seen from the ratio of the increments, Table II. If the hæmolytic indices of mixtures containing a high concentration of antilysin such as those investigated above, are approximate measures of the free lysin present, then, for both original and residue, the amount of added lysin left free is roughly proportional to the lysin added. The amount of added lysin left free by the original is approximately double that left free by the residue, which agrees with the conclusion that the free antilysin in the latter is present in greater concentration than in the former.

On the Reversibility of the Reaction.—The reversibility of the reaction will be demonstrated if, on removing the free lysin, the compound of lysin with antilysin dissociates, producing more free lysin; on the other hand, if no lysin be set free, the reaction is irreversible. In the case of the reaction being reversible, the velocity of dissociation will probably increase rapidly with rise of temperature. A temperature of 37° C. was employed as more likely to show reversibility if such exists.

The methods employed were: (*a*) filtration through gelatine; (*b*) diffusion through gelatine.

(a) *Filtration through Gelatine.*—The practically constant concentration of free lysin in the residues of Experiments Nos. 5 and 6, Table I, might well be due to reversibility. To test this the residue of No. 4 (index 31·25) was diluted with saline to the volume of the original mixture, and heated for $2\frac{1}{2}$ hours at 37° C. On again filtering through gelatine until the new residue had the same volume as the first residue, the hæmolytic index was found to be (27·8).* On repeating this treatment the hæmolytic index was found within the experimental error unchanged. The gelatine obtained from the filters used was strongly hæmolytic (index 100). If the free lysin of the first residue had alone been available, viz., $33 \times 31{\cdot}25 = 1031$ "hæmolytic units," then as filtration removes 300 or more "units," the second residue could not have had a higher index than $\frac{1031-300}{32{\cdot}5}$ = (22·5) and the third residue (13). It would appear then that a portion of the apparently combined lysin has become free on heating at 37° C.

I conclude, therefore, that *in a neutral mixture the reaction is at* 37° *C. at least partially reversible.*

(b) *Experiments on the Diffusion through Gelatine of Lysin and Antilysin.*—Dr. G. Dean kindly suggested to me a suspension of red blood corpuscles in gelatine as a convenient indication of the diffusion of lysin. In applying this method I found it was necessary to allow the lysin to diffuse from a solid gelatine layer into the solid gelatine suspension, for when normal saline alone is placed above a 2·5-per-cent. suspension of corpuscles in gelatine (9 per cent.), hæmolysis occurs. This proceeds from the surface downwards at a rate which is not altered when a strong fluid lysin is used instead of the saline. To avoid this effect, which is probably an imbibition phenomenon, the precipitated lysin was dissolved in 9-per-cent. gelatine at about 37°, and poured on to the strongly cooled columns of 9-per-cent. gelatine containing corpuscles. A rate of hæmolysis was then obtained which decreased with decreasing concentrations of lysin, and was entirely absent when saline in gelatine was used. The course of the diffusion is evidenced by a zone of transparent gelatine coloured with the freed hæmoglobin and a second zone in which partial hæmolysis has taken place, which latter has a fairly sharp boundary against the unhæmolysed and turbid suspension. The experiments were carried out at about 18° C. The gelatine suspensions were contained in tubes of 1 cm. diameter, and were 10 cm. in length. The test volume of supernatant fluid was 1 c.c.

---

* *Cf.* Table I, No. 4A.

Lysin.—The rate at which hæmolysis proceeded from the surface decreased when the concentration of the lysin decreased from 1 to 0·0625 per cent., but lower concentrations down to 0·0078 per cent. gave almost the same effect as 0·0625 per cent. *For low concentrations, then, the rate of the hæmolytic effect is not dependent on the mass of the lysin.* Controls with saline gave no trace of hæmolysis.*

Antilysin.—A layer of the gelatine blood suspension 2 mm. thick was placed on a gelatine column containing 10 per cent. antilysin, and after standing for 40 hours at 18° C., a gelatine layer containing 0·5 per cent. lysin was superimposed. The blood was completely hæmolysed in 40 hours, whereas in a control with gelatine saline no hæmolysis took place. When, however, antilysin was mixed with the gelatine suspension, so that the concentration was 2·5 per cent., it was found that a gelatine layer containing 0·5 per cent. lysin failed to give a trace of hæmolysis in 40 hours. It follows, therefore, that the antilysin in the first experiment could not have been present in a concentration of 2·5 per cent. even in the layer immediately in contact with the 10-per-cent. antilysin in gelatine.

Again, as 0·5 per cent. lysin hæmolyses 2 mm. in 40 hours under normal conditions, no retardation has taken place, and it is probable that *megatherium antilysin does not diffuse appreciably through gelatine.*

Mixtures.—A neutral mixture, containing 0·5 per cent. precipitated lysin and 3·73 per cent. antilysin in saline gelatine (Table IV, No. 2), *and an over-neutralised mixture,* No. 1, containing 0·5 per cent. lysin and 5 per cent. antilysin, after being brought to equilibrium, *showed the presence of at least a trace of free lysin.* The hæmolytic effects were equal and of the magnitude for which

Table III.—Diffusion of Megatherium Lysin through Gelatine Columns, showing Hæmolysis, in Millimetres.

Percentage of lysin ……	1	0·5	0·25	0·125	0·0625	0·0317	0·0159	0·0078	Saline controls.
40 hrs. ………	2·6	2·0	1·7	1·4	1·0	1·0	1·0	1·0	0·0
70 " ………	4·5	3·5	2·2	1·5	1·0	1·0	1·0	1·0	0·0

---

* Compare Table III.

Table IV.—Diffusion of Lysin (0·5 Per Cent.) with Antilysin after attaining Equilibrium, through Gelatine Columns, showing Hæmolysis, in Millimetres.

Percentage of antilysin	No. 1. 5	No. 2. 3·73	No. 3. 2·5	No. 4. 1·25	No. 5. 0·625	No. 6. 0·317	Saline controls.
40 hrs.	tr.	tr.	tr.	tr.	1·4	2·0	0
70 „	1·0	1·0	1·0	1·0	1·5	3·5	0

no proportionality had been found to exist between mass of lysin and effect. It is remarkable that although in 70 hours this effect was evident, yet in less than 40 hours the corresponding free lysin had a greater hæmolytic effect. This seems most easily explained on Ehrlich's view that *lysins are complex and that antilysin will neutralise the most active constituents first.* The same effects were obtained from mixtures of lysin and antilysin in the same relative proportions, but present in ½, ¼, and ⅛th of the concentration obtaining in the above experiment. Controls with saline showed no effect. The partially neutralised mixture containing 0·5 per cent. lysin and 2·5 per cent. antilysin No. 3, gave the same effect as the neutral mixture and likewise a mixture of 0·5 per cent. lysin and 1·25 per cent. antilysin, No. 4, but when the antilysin concentration was reduced to 0·625 per cent. (No. 5), the effect indicated a concentration equal to 0·125 per cent. free lysin. With 0·3175 per cent. antilysin (No. 6), and less the effect indicated 0·5 per cent. lysin free.

These results indicate that *the addition of antilysin up to* 1/10*th the amount required to entirely prevent hæmolysis* at 37° in the standard time (neutral mixture) *does not appreciably neutralise the lysin.* That the combination of lysin with antilysin is easily reversible at 18° C., does not seem probable, for on the addition of double the amount of antilysin the free lysin decreases to less than 1/4th of the total amount added. This then apparently conforms well with Ehrlich's view that no neutralisation of toxin takes place when small quantities of antitoxin are added.

On the Nature of the Equilibria: Fractional Addition of Lysin to Antilysin.

Danysz (1902) found that when a certain quantity of diphtheria toxin was added in fractions to antitoxin, less toxin was neutralised than in the case in which the whole quantity was added in one portion. This result has been confirmed by von Dungern (1904). Dr. C. Todd first drew my attention to the fact that false equilibria were attained on the fractional addition of megatherium lysin to antilysin (experiments unpublished).

On further investigation I found, however, that the nature of the equilibria obtained in partially neutralised and in neutral mixtures is not the same. This may be seen from the following experiments:—

One c.c. of 0·625 per cent. antilysin was placed in each of two series of tubes. To one series 1 c.c. of various dilutions of lysin was added, and the mixture heated for two hours at 37° C. To the other series 0·5 c.c. of the same dilution of lysin was added, and the mixtures heated for one hour at 37° C. Another similar addition of 0·5 c.c. was then made, and the mixtures heated one hour at 37° C. Table V shows that the mixtures which were made in this intermittent way have higher hæmolytic indices than the mixtures which were made at one operation. The above was only found to be true when the resulting mixtures were strongly hæmolytic. When, however, the mixtures contained considerably less free lysin, the hæmolytic indices were the same in both series. From this and similar experiments, in which the volume of the antilysin was not appreciably modified by the addition of the lysin in concentrated form, it follows that false equilibria are attained in the lysin-antilysin reaction when the final concentration of the free lysin is high. In more nearly neutral mixtures

Table V.

Addition at once.			Fractional addition.				
2 hrs. at 37°.			Part I, 1 hr. at 37°.		Part II, 1 hr. at 37°.		
Added to 1 c.c., 0·625 per cent. antilysin.					Added to Part I.		
Lysin in c.c.	Saline in c.c.	Hæm. index.	Lysin in c.c.	Saline in c.c.	Lysin in c.c.	Saline in c.c.	Hæm. index.
0·1	0·9	18·3	—	—	—	—	—
0·2	0·8	20·8	0·1	0·4	0·1	0·4	18·3
0·3	0·7	24·6	—	—	—	—	—
0·4	0·6	31·7	0·2	0·3	0·2	0·3	32·1
0·5	0·5	39·2	—	—	—	—	—
0·6	0·4	48·3	0·3	0·2	0·3	0·2	60·0
0·7	0·3	71·2	—	—	—	—	—
0·8	0·2	91·7	0·4	0·1	0·4	0·1	100
0·9	0·1	98	—	—	—	—	—
1·0	0·0	100	0·5	0·0	0·5	0·0	100

an apparently true equilibrium results. From Table V the irreversibility in nearly neutral solutions gives a slight effect in the opposite direction to the effect obtained by Danysz and von Dungern.

On the Mass Action of Lysin and Antilysin.

If the equation, toxin + antitoxin = 2 (toxin, antitoxin), proposed by Arrhenius and Madsen, holds for lysin and antilysin, then, when the same relative concentrations of these bodies are brought together, the equilibria attained will be the same, *i.e.*, the relative concentrations of the substances will be independent of the volume of the mixture. Thus, 10 c.c. of a mixture of lysin and antilysin in equilibrium will contain as much free lysin as 1 c.c. of a mixture 10 times as strong in total lysin and antilysin.

In Table VI (*a* 1, 2) and (*a* 5, 6) are such mixtures which were brought to equilibrium at 37°, the test volume (3) of the first mixture was 1 c.c., and of the other 0·1 c.c., added with 0·9 c.c. saline (7). The hæmolytic indices (4) (8) are not equal, being respectively (50) and (60).

Table VI.—Hæmolytic Indices of Multiple Mixtures of Lysin and Antilysin in Equilibrium.

	1.	2.	3.	4.	5.	6.	7.	8.
	Lysin conc. per c.c.	Antilysin conc. per c.c.	Test volume in c.c.	Hæm. index.	Lysin conc. per c.c.	Antilysin conc. per c.c.	Test volume in 1 c.c.	Hæm. index.
a	0·005	0·01	1·0	50	0·05	0·1	0·1	60
b	0·0025	0·005	1·0	26	0·025	0·05	0·1	50
c	0·0005	0·001	1·0	0	0·05	0·1	0·01	42·5
d	0·00025	0·0005	1·0	0	0·025	0·05	0·01	41

Two similarly related mixtures of half the strength (*b* 1, 2) and (*b* 5, 6) showed a more marked difference, viz. (26) and (50). When the strength of the mixtures were widely different as 1—100, the difference is extremely marked, *e.g.*, (*c* 1, 2) and (*c* 5, 6) gave the indices (0) and (42·5) when 1 c.c. and 0·01 c.c. with 0·99 c.c. saline were used as the test volume respectively. Two similarly related mixtures of half the strength (*d* 1, 2) and (*d* 5, 6) showed a similar effect and indices (0) and (41).

It must then be concluded that Arrhenius and Madsen's equation does not apply to the lysin-antilysin reaction so far as megatherium lysin is concerned.

The Validity of the Application of Chemical Mass Action Equations to the Lysin-Antilysin Action.

Nernst (1904) has insisted that the views of Arrhenius and Madsen on the mass action of toxin and antitoxin can have no true theoretical foundation. If the reaction consist in a chemical change, it seems to me that the chemical law of mass action would be applicable in the manner given below. If, on the other hand, the observed mass action differs widely from the deduced, we can conclude that the toxin-antitoxin reaction is not a purely chemical one.

The anti-body, *e.g.*, antilysin, is a typical colloid, and its active chemical mass will, like that of a solid suspension, be constant. Similarly, the chemically active mass of the compound between antilysin and lysin is constant, as this also occurs in the form of a fine suspension. The active mass of the lysin will vary with its concentration, as this substance is not present in the form of a typical colloid. It has properties such as diffusibility, and the power of passing a gelatine filter similar in magnitude to those found for crystalloids and inferior colloids.

These conditions are not unlike those studied by Walker and Appleyard (1896) in the case of a solid suspension of diphenylamine in picric acid solutions. If this analogy is a true one, the chemical equation expressing the combination of lysin with antilysin should be similar to that given by these authors for the combination of picric acid with diphenylamine, viz., lysin, water + antilysin = compound + water.

The lysin is removed by the solid suspension of antilysin, and a solid suspension of compound (lysin, antilysin) is produced with the liberation of the water which held the lysin in solution. The velocity of combination will be K_1CK' where K_1 is the velocity constant, C the concentration of the lysin, and K' the constant active mass of the antilysin. When the reaction is reversible, *i.e.*, possibly at high concentrations of antilysin, the velocity of dissociation will be $K_2K''K'''$ where K_2 is the velocity constant, K'' the constant active mass of the compound, and K''' the constant active mass of the water. For equilibrium $K_1CK' = K_2K''K'''$, or

$$C = \frac{K_2K''K'''}{K_1K'} = \text{constant}.$$

From which it follows that at any definite temperature the concentration of the lysin must be a constant. The compound (lysin, antilysin) and free antilysin can only exist side by side in the aqueous medium when the latter has a certain fixed absolute concentration. The relative concentrations of free antilysin and compound have no influence on the equilibrium. Thus Walker and Appleyard found that the concentration of aqueous picric acid in contact with varying amounts of diphenylamin is constant, but that the latter was stained more deeply when present in smaller quantities. The relations observed between lysin and antilysin are, however, totally different, for in this case the amount combined varies continuously with the concentration of the lysin.

I therefore conclude that *the removal of lysin from a solution by antilysin is not capable of interpretation as a purely chemical change, and the law of chemical mass action does not apply when the lysin is present in excess.*

From the filtration experiments with neutral and overneutralised mixtures it, however, seems possible that when excess of antilysin is present, the chemical law of mass action holds, for the concentration of free lysin was found to be practically constant when the concentration of antilysin was largely increased.

The application of Adsorption and Surface Tension hypotheses to the Lysin-Antilysin Action.

Walker and Appleyard also investigated the phenomena of the fixation of picric acid by silk, to which the relations of lysin to antilysin, which have been described above, present a much closer analogy. They found that the concentration of the dye bath varies continuously with the depth to which the silk is dyed, and that the concentration of picric acid (C_2) in the silk was proportional to the concentration of free picric acid (C_1) raised to a constant power n, or $C_2 = KC_1^n$. The adsorption of substances, *e.g.*, of iodine from solutions by charcoal (Schmidt, 1894); of iodine from water by starch (Küster, 1894), etc., have in general been found to obey similar relations. It is, then, not improbable that some such adsorption formula may be found to hold for the fixation of lysins, etc., by their respective antibodies, as I suggested in a recent paper on the phenomena of agglutination (1905). I am at present engaged in further investigating the phenomena of lysin-antilysin relations from this point of view, and, so far, the results have been encouraging.

SUMMARY OF CONCLUSIONS.

1. Megatherium lysin passed through a gelatine filter, and is diffusible through gelatine.

2. Megatherium antilysin does not pass through a gelatine filter, and is not appreciably diffusible through gelatine.

3. The filtration and diffusion of mixtures show that free lysin is present in neutral mixtures and in mixtures containing excess of antilysin.

4. Free antilysin exists in neutral mixtures, and in mixtures containing excess of lysin.

5. The reaction is at least partially reversible when excess of antilysin is present.

6. False equilibria are produced with greater facility when the lysin is in excess.

7. The neutralisation equation of Arrhenius and Madsen does not hold for multiple mixtures.

8. The removal of lysin from a solution by antilysin is not capable of interpretation as a purely chemical change, but is more analogous to certain adsorption phenomena.

REFERENCES.

Arrhenius and Madsen (1902). 'Festskrift ved Indvielsen af Statens Seruminstitut,' III.
Arrhenius and Madsen (1904). 'Bulletin de l'academie royale des sciences de Danemark,' p. 271.
Bordet (1903). 'Annales de l'Institut Pasteur,' vol. 17, p. 161.
Craw (1905). 'Journal of Hygiene,' vol. 5, p. 115.
Danysz (1902). 'Annales de l'Institut Pasteur,' vol. 16, p. 331.
Von Dungern (1904). 'Deutsche Med. Wochenschrift,' vol. 30, pp. 275, 310.
Ehrlich (1898). 'Deutsche Med. Wochenschrift,' vol. 24, p. 597.
Ehrlich (1903). 'Berliner Klin. Wochenschrift,' vol. 40, pp. 793, 825, 848.
Küster (1894). 'Liebig's Annalen der Chemie,' vol. 283, p. 360.
Martin, C. J. (1896). 'Journ. of Physiol.,' vol. 20, p. 364.
Martin and Cherry (1898). 'Roy. Soc. Proc.,' vol. 63, p. 420.
Nernst (1904). 'Zeitschrift für Elektrochemie,' vol. 10, p. 377.
Reid, E. W. (1901—2). 'Journal of Physiology,' vol. 27, p. 161.
Schmidt (1894). 'Zeitschrift für Physikalische Chemie,' vol. 15, p. 56.
Todd (1902). 'Trans. of the Pathological Society of London,' vol. 53, Part II, p. 196.
Walker and Appleyard (1896). 'Journal of the Chemical Society,' vol. 69, p. 1334.

*Two Cases of Trichromic Vision.**

By F. W. Edridge-Green, M.D., F.R.C.S.

(Communicated by Dr. F. W. Mott, F.R.S. Received January 24,—Read February 23, 1905.)

1. One case (Professor J. J. Thomson) sees only three colours in the bright spectrum—red, green, and violet. He can distinguish nothing of the nature of pure yellow, like the sensation given him by the sodium flame in the spectrum. There is no definite colour to him at the portion of the spectrum where the normal sighted see pure blue. Reddish-green would describe the orange and yellow regions and greenish-violet the blue. λ 5950 (orange-yellow) is the point which differs most from red and green. There was no shortening of either end of the spectrum. The point of junction of the red and green differed somewhat in repeating the observations because of his great sensitiveness to simultaneous contrast. It was, however, always in the orange or orange-yellow, never in the yellow of the normal sighted.

Difference of Hue Perception.—I then tested him with my apparatus for ascertaining the size of different parts of the spectrum which appear monochromatic, and found that he was defective in distinguishing differences of hue. A portion of the spectrum corresponding to the D lines, and isolated by two shutters in the eye-piece of the spectroscope, was first shown. The shutter on the red side was gradually opened until a difference of hue was seen. The monochromatic patch extended from λ 5889 to λ 6052, being exactly half as large again as that of the normal sighted, which occupies the space from λ 5889 to λ 5998. The monochromatic patch he called greenish-yellow. His monochromatic patch in the centre of the green bore exactly the same proportion to mine as in the case of the orange-yellow, being just half as large again.

Colour Mixtures.—He was then tested with Rayleigh's apparatus for matching spectral yellow by a mixture of red and green. 0 of the scale corresponded to pure red, 25 or 90° to pure green. He made the following 10 matches:—

---

* This research was made with the aid of an instrument purchased with a grant from the Government Grant fund.

Match.	Difference.	Match.	Difference.
†1. 13·40	+0·67	* 6. 11·75	−0·98
*2. 12·0	−0·73	* 7. 11·75	−0·98
†3. 12·75	+0·02	† 8. 13·50	+0·77
*4. 12·0	−0·73	† 9. 12·90	+0·17
†5. 13·50	+0·77	*10. 13·75	+1·02

Average, 12·73. Average difference, 0·684.

I find that my colour vision agees with that of the large majority of persons and may, therefore, be regarded as normal. I made the following 10 observations for comparison with those given above :—

Match.	Difference.	Match.	Difference.
*1. 11·0	+0·629	* 6. 10·0	−0·371
†2. 10·25	−0·121	† 7. 10·50	+0·129
*3. 9·80	−0·571	* 8. 10·50	+0·129
†4. 10·33	−0·041	† 9. 10·60	+0·229
†5. 10·33	−0·041	*10. 10·40	+0·029

Average, 10·371. Average difference, 0·229.

* Red shown first in the mixed colour. † Green shown first.

My match appeared to him bright red and bright green, the yellow appearing as green. The match appeared more correct through a pin hole. The mixed colour of his match always appeared green to me. It will be noticed that his average difference is very nearly three times the amount of mine. On comparing the differences, according to the colour which was shown first, it will be found that these were all positive when the green was shown first, and four out of the five were negative when he commenced with the red.

Green first	+0·67	Red first	−0·73
	+0·02		−0·73
	+0·77		−0·98
	+0·77		−0·98
	+0·17		+1·02
Average,	0·48	Average,	0·888

Below I give my differences for comparison.

Green first	−0·121	Red first	+0·629
	−0·041		−0·571
	−0·041		−0·371
	+0·129		+0·129
	+0·229		+0·029
Average,	0·1122	Average	0·3458

He seemed to get very easily fatigued with colours.

Classification Test.—Called I (orange) "reddish-orange," and matched it with orange and dark yellows. Described II (violet) as mauve, and put with it violets and purples. Named III (red) correctly, and picked out various reds to go with it. Called IV (blue-green) "green," and matched it with some greens. Only a few colours were selected in each case. On being asked to pick out all the yellows he chose those with orange in them. He regarded orange-yellow as his yellow and rejected pure yellows because he said that they had green in them. He had considerable difficulty in matching the colours. In common with the cases I have previously observed, the effects of simultaneous contrast were much more marked than in the normal sighted. Two wools changed colour to him on being contrasted, when no change was evident to me. This was particularly noticeable when one of the contrasted colours was either red, green, or violet, and the other one of the intermediate and adjacent colours.

Lantern Test.—He correctly named the red, green, and violet with and without the neutral glasses, and saw them at the normal distance. He had difficulty with yellow and blue. He called pure yellow "greenish-yellow."

It will be noticed that the examination with the spectrum gives a key to the mistakes made.

2. The other case is that of Mr. P. S. Barlow, B.Sc., a research student in the Cavendish Laboratory. He sees three colours in the spectrum—red, green, and violet. The red gradually passes into the green, and red-green would describe this region and green-violet that of the blue. He sees no yellow or blue in the spectrum. When I put the pointer in the yellow he said it was in the green. He gave λ 5892 three times out of four as the junction of the red and green, the fourth time λ 5950. Both are in the orange-yellow. He selected λ 4800 (blue) as the point of union of the green and violet. He was very sensitive to simultaneous contrast. When shown the violet first he put the junction of the green and violet at λ 4861; when shown the green first at λ 4740. There was no shortening of either end of the spectrum.

Difference of Hue Perception.—He was tested in the same way as Professor Thomson. He also called the orange-yellow patch "yellow-green," and I had to increase the size of the patch until it was half as large again (λ 5889 to λ 6052) as that of the normal sighted before a difference was seen. I examined him in the same way with the other colours of the spectrum, and found that in every part he marked out a much larger monochromatic patch than the normal sighted.

Colour Mixtures.—He was then tested with Rayleigh's apparatus. On being shown my match he said that the yellow was green and the mixed colour salmon pink. He said that the yellow of my match was too dark, and in order to make a match, as far as luminosity was concerned, he had to increase the brightness of the yellow. No match was then possible to me. He made the following 10 matches:—

Match.	Difference.
*1. 15·66	−0·815
*2. 17·0	+0·525
†3. 16·90	+0·425
*4. 16·33	−0·145
†5. 17·40	+0·925

Match.	Difference.	Difference, red first.	Difference, green first.
† 6. 17·0	+0·525	−0·815	+0·425
* 7. 16·0	−0·475	+0·525	+0·925
† 8. 15·33	−1·145	−0·145	+0·525
* 9. 15·80	−0·675	−0·475	−1·145
†10. 17·33	+0·855	−0·675	+0·855
Average, 16·475	Average, 0·651	Average, 0·527	Average, 0·775

* Red first. † Green first.

Lantern Test.—He called pure yellow "yellow-green" and pure blue "blue-green." Dark blue he called purple. The other colours he named correctly.

Classification Test.—I he designated "golden yellow" and matched it with orange. II he called "purple" and put with it violets and purples. III he said was "crimson" and sorted out a few reds to go with it. IV he named "blue" and matched with greens and blues. Many colours were omitted. He called yellow "yellow-green." He chose orange-yellow for yellow. He found great difficulty with blue and green. Ladies have several times told him of mistakes in this respect. On being shown IV (blue-green) a second time, he said that it was "pure green without a trace of blue." Simultaneous contrast was very strongly marked.

I use the term *trichromic* as a statement of the fact that persons having this vision see only three colours in the bright spectrum, whilst the normal sighted see six, and may, therefore, be designated *hexachromic*. It is probable that the appearance of the bright spectrum to the trichromic is very similar to that of a spectrum of feeble luminosity to the normal sighted, in which only three colours—red, green, and violet—are seen. The defective difference perception which is found in these cases accounts for most of the facts. Both these cases are bordering on the tetrachromic, as the sodium flame appears to give rise to a distinct sensation.

The Colour-Physiology of the Higher Crustacea, Part III.

By Frederick Keeble, M.A., University College, Reading, and F. W. Gamble, D.Sc., Zoological Department, Victoria University, Manchester.

(Communicated by Professor Sydney J. Hickson, F.R.S. Received February 8,—Read February 23, 1905.)

(Abstract.)

1. The chromatophores of *Hippolyte* and *Crangon* are multicellular structures. Their branches show differentiation into a firmer ectoplasm and a more fluid mobile endoplasm in which the pigment occurs.

2. The formation of the pigments in the larval and post-larval chromatophores is described.

3. In addition to pigments, fat, in the form of colourless globules, occurs in the chromatophores of *Hippolyte.* This fat lies in special cells of the chromatophore, and exhibits a mobility similar to that of the pigments of the chromatophore.

4. If fed and kept in the dark, or if starved and kept in the light, *Hippolyte* loses little of its chromatophoric fat. Depletion of fat occurs, however, in starved, dark-kept, animals. These, when exposed to sunlight for five or six hours, show fat in their chromatophores. These results show that the colourless chromatophoric fat is a reserve food material, and point to the conclusion that in the accumulation of this reserve fat, light plays an important part.

5. At the time of settling on the weeds of the sea-shore, *Hippolyte varians* is a colourless or faintly brown-striped animal. At this stage it is extremely sensitive to the light conditions of its environment, assuming the colour of its surroundings within 24 hours. If the environment be changed, sympathetic change of colour takes place in three days. Half- and full-grown *Hippolyte* are less susceptible. With them sympathetic colour-change occupies a week or more.

On Colour-Vision by Very Weak Light.

By George J. Burch, M.A., D.Sc. Oxon.; F.R.S.; Lecturer in Physics,
University College, Reading.

(Received March 7,—Read April 13, 1905.)

In my paper on Artificial Temporary Colour-Blindness, I wrote:—"Hering's black-white sensation I have not found, but the evidence for and against it is of a somewhat different character, and I propose to discuss it in a separate paper."

Hering's theory, as is well known, rests partly on the statement that, under a very feeble illumination, all colours appear grey—that there is an interval between the chromatic threshold and the absolute threshold of light sensation—and his statement is supported by a number of experiments by various observers, which have been received as classical.

I find myself under the necessity of traversing this statement, inasmuch as in my own case, to the perfectly rested eye, red, green, blue, and violet are instantly recognised as colours and distinguished from each other, and there is no interval between the chromatic and the absolute threshold, and it is only when the eye is not perfectly rested that all colours appear grey in a feeble light.

In laying before the Society an account of the experiments which have led me to this conclusion I have endeavoured, in view of the direct conflict between my testimony and that of so many previous writers, to give sufficient details to justify my own position, and to indicate a possible explanation of these contradictory results.

Early Experiments.

Experiment 1.—The mental attitude of the observer, of comparatively little interest in other cases, has a certain importance when subjective phenomena are under investigation. For this reason my first experiment is described in greater detail than would otherwise be given.

I had been repeating, in the course of my studies, and with some success, as many as possible of the experiments referred to in my books. Among others was the statement that, with sufficiently feeble illumination, all colours appear grey. In order to observe this, I arranged a metal chimney, furnished with a cap, over a bunsen burner so as to completely prevent the escape of light without interfering with the free supply of air. By bringing the flame near the side of the chimney, and increasing or diminishing the supply of gas, the metal could be brought to a dull red heat only just visible.

Operating at night in a room with the blinds drawn, the first appearance of luminosity was of a pearl-grey tint. Encouraged by this result, I proceeded to try how much fainter the light could be made in an absolutely dark room without becoming invisible. Accordingly, my father had the gas laid on in an inner room which had no windows. The bunsen burner, standing in the middle of the metal chimney, was lit and turned down low. After remaining in complete darkness for two hours I moved it to the side, so that the flame touched the metal, and waited. To my surprise, the first visible luminosity was dull red, instead of grey as in the previous experiment. Slightly reducing the gas supply caused it to disappear gradually, but without passing through grey.

It should be noted that I was disappointed at having obtained, after so much trouble, what seemed a worse result, and ascribed it to a too rapid raising of the temperature, but was unable by the most careful adjustment of the lamp to see any traces of the light before it appeared red. The next night, after repeating the experiment with no greater success, I went to get a screw-clamp from the outer room, which was dark save for the light of a street lamp 100 yards away, upon the canvas sun-blind, which was drawn down. After returning, the first visible luminosity was grey until I had remained in darkness another half-hour, when I could see only red, as before. My original bias being opposed to the result obtained, gives greater weight to the experiment.

In order to have another colour for comparison, I drilled a small hole in the back of the chimney on a level with the flame, the blueish light of which, received on a sheet of paper about 18 inches off, was just visible. This appeared of a rich blue tint, in strong contrast to the dark red of the metal. By varying the distance of the paper from the chimney the relative intensities of illumination could be adjusted until both reached the minimum simultaneously. But the contrast of colour was always visible, however faint the light, *when the eye was completely free from the after effects of previous stimulation.*

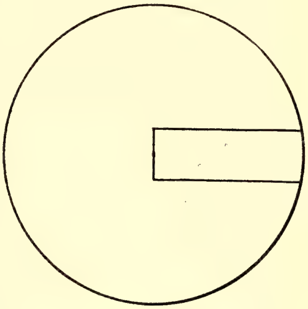
Experiment 2.—Glowing metal did not seem satisfactory as a source of light, because the refrangibility of the rays increasing continuously with the temperature, there must be a quantity of green rays almost capable of exciting sensation by the time the red rays were strong enough to do so. I therefore modified the experiment, using the spectra of rarefied gases because it is easier to distinguish differences of colour between two parts of a spectrum that is not continuous.

The method, which I finally adopted, of reducing the intensity of the illumination to the requisite degree, was to vary the distance between the spark-gap, or vacuum tube, and a piece of white paper, towards which the slit of the spectroscope was directed, the whole apparatus being in the dark room, and enclosed in a box blackened inside and covered with a black cloth, through which the eyepiece of the spectroscope projected.

For bright-line spectra, it is better to have the slit rather wide. It is difficult, after staying some hours in total darkness, to tell exactly how the eye is focussed when straining to catch sight of an almost invisible object, and the intensity of the stimulus due to a small object is much diminished when the image of it, being out of focus, is spread over a larger surface of the retina. As soon as the object is caught sight of, the difficulty vanishes and the slit may be narrowed.

To locate the position of a given line, I used a broad pointer with a square end, fixed horizontally in the focal plane of the eye-piece (Fig. 1). This causes an easily visible gap to appear and disappear as it is moved to and fro over a faint line.

Fig. 1.



With this arrangement I found that lines as far in the red as that of lithium always appeared red, however faint, if the eye was sufficiently rested, and that the sodium lines were green, but that, with more complex spectra, the middle portions were so much brighter that it was difficult to see the ends. I therefore prepared a light-filter, composed of gelatine films stained to the requisite depth with aniline and other pink and purple dyes, painted on with a brush until the faintest visible spectrum of lamplight, seen through them, appeared no brighter at the middle than at the ends.

Experiment 3.—Using the above described light-filter, I placed in the focal plane of the eyepiece a stop with four slits so spaced as to leave visible a strip of red near B, of yellowish-green near D, of blue close to F, and of violet as far beyond G as the light employed would admit. I found afterwards that a small strip of paper covered with Balmain's Luminous Paint, aid partly across the white reflecting surface, would supply additional violet if necessary.

The illumination was made so faint that even after two hours in tota darkness I could not see the bands continuously, but could only catch glimpses of them from time to time, as one does when looking for stars in early twilight. It is very difficult to tell in total darkness in what direction to look, even with the eyebrow touching the eye-piece, so that the band first seen was seldom the one I expected to find. Yet the colour was instantly recognised.

Each colour produces a characteristic effect quite apart from tone or tint. The red band catches the eye suddenly as it sweeps to and fro through the darkness. Once seen, it is easily held for some seconds—and lost as easily. Its edges are perfectly definite against the darkness.

The blue band first shows as a blue haze, persistently appearing in one spot, and refusing to follow the eye as it sweeps across. On looking steadily the haze resolves itself into a band with fairly definite outlines. (It should be noted that the method adopted, of reducing the intensity of the light *before it enters the spectroscope*, effectually does away with the false light that is so troublesome in some cases. There is no visible "field of view.")

Violet appears first as a haze of violet after-effect, which seems to have a cumulative action with regard to the sensation it excites. It is difficult to see the outline of the band, owing apparently to its strong tendency to produce after-effects even with such low intensity. But if the eye is kept perfectly still the violet is held, when once found, even more easily than the red.

There is a curious phenomenon associated with the green that I do not understand, but have seen so often that I here call attention to it. The outline is definite though not so definite as that of the red, but the band seems to sparkle as I look at it. That is to say, it is not continuously equally bright all over, but spots of slightly greater luminosity of perhaps 5′ or 8′ diameter appear and disappear in various parts of it. Any portion of the spectrum from D to *b* will do this, but beyond *b* the steady light of the blue sensation seems to mask the effect, which is only visible when the eye is completely rested (after two hours darkness at least) and with very faint illumination—either the minimum visible or a little more.

Experiment 4.—This experiment is the converse of the preceding. I arranged a weak continuous spectrum of lamplight which, after half-an-hour's stay in darkness appeared white, or rather colourless, from end to end. After setting the broad pointer to mark the extreme limit of this spectrum towards the red, I took away the paper reflector and put the lamp in its place. What I had taken for the extreme limit of the spectrum proved to be the beginning of the red. I concluded, therefore, that the ratio of the light sensation to the physical stimulus varies differently with the intensity for red and for green, and that it was desirable to make quantitative measurements.*

With respect to the violet end of the spectrum another difficulty arises. Paper of all kinds fluoresces strongly in the violet, giving out a light consisting largely of green, and is hence unsuitable as a reflector before the slit of the spectroscope to reduce the light. Porcelain, which does not fluoresce, does not seem to reflect much of the violet near H and K. Powdered sodium bicarbonate, pressed into a flat cake, seems as good as anything if pure.

This part of the work was done from 1873 to 1878 in my father's works at Cheshunt.

Later Quantitative Experiments.

I repeated these experiments in 1893–5 with precisely similar results, in the Physiological Laboratory, Oxford, but on referring to the literature of the subject and finding how many experiments had been published with results diametrically opposed to mine, I determined to investigate the subject yet a third time, and to obtain also quantitative measurements in regard to: (*a*) the sensitiveness of the eye to red and to blue by daylight and in darkness, and (*b*) the time-relations of the subjective phenomena which accompany a prolonged period of darkness.

This final revision of the work was begun in 1901 and, after various unavoidable delays, was completed about a month ago.

The source of light was a 16 candle-power glow-lamp placed some distance from a piece of white paper fixed at an angle of 45° outside the wall of the dark room, in which was a circular aperture 2·5 cm. in diameter. The arrangement is shown diagrammatically in Fig. 2. A is the glow-lamp, B the paper reflector, C the wall of the dark room, D a polarising prism, E the spectroscope, with F a double-image prism over the eye-piece.

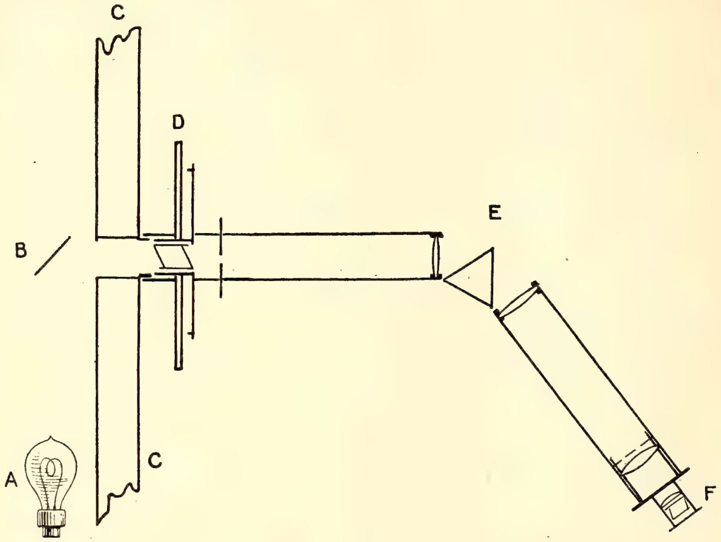
In the focal plane of the eye-piece I placed a stop with two narrow slits, the distance between them being such that if the red about B was brought under one, the blue-violet about G was visible through the other. With the double-image prism the order in which the bands appeared was V_1, V_2, R_1, R_2.

Blue-violet was chosen to contrast with red because the light available was not strong in violet and I considered the relative intensity of the red and this part of the spectrum might be less affected by changes in the strength of the current. For the same reason I used paper instead of sodium carbonate, thus getting rid, by fluorescence, of a great part of the violet rays.

---

* I was not at that time acquainted with Purkinje's work, with which this is in entire agreement.

Fig. 2.



The experiment consisted in turning the polarising prism D until the blue-violet band V_2 and the red band R_1 appeared equally bright, and then altering the intensity of the light by placing the glow-lamp A nearer to or farther from the paper reflector B, and finally, if any change was observed, readjusting the polarising prism until V_2 and R_1 were again equally bright. From the data thus obtained, the relative intensities of the colour-sensations under the different degrees of illumination could be calculated.

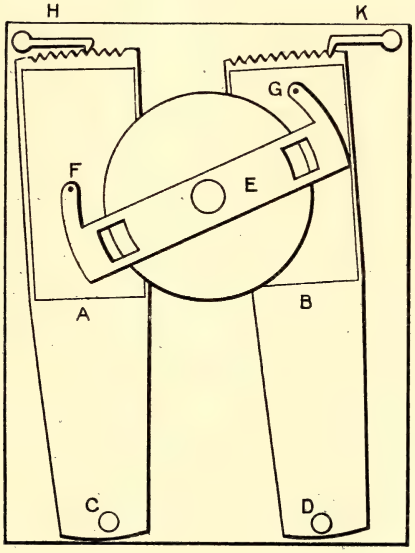
Here some explanation is necessary. It may be objected that there can be no definite standard of equality of brightness between two different colours. That is perfectly true in the sense that not only does the position of the prism vary when it it is set by different observers, but the same observer, under different conditions of light adaptation, makes very different settings. What I here mean by "equality of brightness" between two colours is this: an observer sets the prism so that the two colours are, in his opinion, equally bright. The prism is then displaced by someone else, and the observer asked to repeat the setting immediately. He will probably do so with not more than 2 per cent. variation in the relative intensity, and generally much less. His standard of equality is personal and empirical, but it is constant so long as his physiological condition is unchanged.

It would seem that after remaining in total darkness for two hours, a fairly constant physiological condition is reached, and I have, therefore, compared all the ratios with those obtained under these circumstances.

It should be noted moreover, that the feebler the sensation the less the judgment of the observer enters into the result, until finally, when both bands are so faint that they can only just be glimpsed from time to time, the sensations may be considered equal if each is seen with equal difficulty.

In order to record a series of observations made during a long period of darkness, I devised the simple arrangement shown in Fig. 3.

FIG. 3.



The divided circle is fixed about 5 mm. in front of a larger square plate on which two thin pieces of wood A and B, are pinned by the pivots C and D. Each piece is furnished with a notched sector of sheet brass in which the spring catches H and K engage. The brass plate E fixed to the tube containing the Nicol prism carries, in addition to the verniers, two thin springy strips of sheet brass F and G. To the end of each strip is soldered a small drawing pin carefully sharpened, and a thin card is fastened to each of the pieces A and B.

After the first observation made in the dark room the strip F is pressed so that the point marks the card. A is then moved one notch so that the mark for the next observation may come on a different part of the card. I have found it convenient to use the other spring G for recording results obtained at the end of the period of darkness. As a check, the two positions of extinction are marked with both F and G.

After coming out of the dark room the pins are carefully adjusted to each of the marks in succession, using a magnifying lens, and the corresponding readings taken. As there is no possibility of mistaking the order in which the marks were made, owing to the cards having been moved on between each observation, the Nicol may be turned back some 20° or 30° so that there may be nothing to prejudice the observer in his next attempt. Practically I found this made no difference and is not advisable in dealing with very feeble illumination, because it exposes the eye to an image which may be several times stronger than the minimum visible.

Experiment 5.—Made at night, after walking a mile and then spending half-an-hour in the gas-lit laboratory.

Having arranged the apparatus with the room lighted, I reduced the illumination of the spectrum to the minimum visible and adjusted the Nicol till the red R_1 and the blue-violet V_2 were equally bright, and pricked a record. I then moved the lamp A (Fig. 2) so much further from the paper reflector that I could see nothing whatever in the spectroscope, shut myself in the dark room, turned out the lights, and waited. After 15 minutes I could just detect the blue-violet band, and after 30 minutes the red also was visible, the blue appearing most luminous. I then adjusted the Nicol until both were equally bright, and pricked the record.

The first record was $13^\circ\cdot7$ and the second $37^\circ\cdot2$ from the position of extinction of the red. The intensities of the two images were, therefore:—

$$V_2D_1^{-2}\cos^2 13\cdot7 : R_1D_1^{-2}\sin^2 13\cdot7 \text{ in the first case,}$$
$$\text{and } V_2D_2^{-2}\cos^2 37\cdot2 : R_1D_2^{-2}\sin^2 37\cdot2 \text{ in the second,}$$

where D_1 and D_2 are the distances of the lamp A from the paper in the first and second cases respectively. That is to say, after half-an-hour's rest in darkness the sensitiveness of the eye had increased to red, and had increased also to blue-violet, but in so much greater proportion that it now required 9·7 times as much red to match the feeblest visible blue as it did before the eye was rested.

Experiment 6.—Made on a dull day after a walk of three-quarters of a mile, followed by half-an-hour in the laboratory.

The Nicol was adjusted till the red and the blue-violet appeared equally bright, all the blinds being up and the gases lit. The intensity was just too low to enable me to see the cross-wires. Taking this ratio as unity, at the end of 5 minutes the red had to be increased to 6 times, and after 20 minutes to 7·6 times its previous amount, to match the blue-violet. With a weaker light 11·27 times the amount of red was required, and after 45 minutes, with a still weaker light, 16·82 times the original proportion of red was required to match the blue-violet.

This explains, I think, why the yellow of the spectrum shifts so much towards the red end with very weak light after a prolonged stay in darkness.

Experiment 7.—In order to compare measurements made with light of equal intensities during the progress of the change, and after the eye had reached the constant condition, I arranged to remain continuously in the dark room while an assistant came at stated intervals and moved the lamp from one to another of a series of increasing distances from the paper screen. Each time I marked the position of the prism for which the red and the blue-violet appeared equally bright, and the final distance was the greatest at which I could distinguish the two bands.

At the end of 45 minutes it was found necessary to bring the experiment to a conclusion before reaching the final stage of adaptation. The assistant, therefore, moved the lamp to each of the fixed distances in the reverse order, and I took a record, as quickly as possible, of the setting of the Nicol for which the colours appeared equal in brightness. The width of the slit remained the same throughout, having been adjusted at the commencement of the experiment so that the bands were, to the non-rested eye, of minimum visible luminosity when equal in brightness.

In the table "Time" signifies the number of minutes after the room was darkened. "Intensity" represents the physical intensity of the band in

During the Process of Resting in Darkness.

Time.	Intensity of red.	Intensity of blue-violet.	Ratio.	
0	3·17	103·28	31·83	Minimum visible.
5	4·34	23·49	5·41	Easily visible.
20	1·20	5·80	4·84	After blow on the eye.
30	1·14	2·19	1·93	Easily visible.
45	1·00	1·00	1·00	Minimum visible.
After Resting in Darkness.				
45	1·00	1·00	1·00	
46	1·34	2·06	1·53	
47·30	1·98	5·30	2·67	
49·30	4·97	23·08	4·64	
51	11·55	97·67	8·46	

terms of its intensity when the lamp was at the greatest distance from the paper reflector, namely, 2 metres. "Ratio" is the quotient of the intensity of the blue-violet band by that of the red, and shows the proportion of blue-violet to red necessary to produce an equal sensation under the conditions of the experiment, taking as unity the ratio for the minimum visible to the rested eye.

Although the positions in which the lamp was placed for the second set of readings were the same as for the first set, the intensities are different, because in adjusting the two colours to equal luminosity by the Nicol prism, the one is increased and the other diminished. This is one of the drawbacks of the polarisation type of photometer.

The third measurement is interesting.

Moving my head towards the instrument in the dark, the cap of the eye-piece came sharply in contact with my face close to the eye. The bands, which had appeared red and blue, though barely visible, were instantly obscured by a luminous fog of after-effect, so that I could not take the reading for some 30 seconds, and when I did, they both appeared colourless.

Experiment 8.—In this case I remained more than two hours in total darkness, but did not take the first record till nine minutes had elapsed. The unit of intensity is the minimum visible at the end of two hours, *i.e.*, with the lamp 3 metres from the paper. The slit was, I believe, narrower than in the preceding experiment.

During the Process of Resting in Darkness.

Time.	Intensity of red.	Intensity of blue-violet.	Ratio.
9	16·63	254·76	15·32
21	11·14	57·61	5·17
25	4·61	12·82	2·78
60	2·40	5·39	2·24
120	1·00	1·00	1·00
After Resting Two Hours in Darkness.			
120	1·00	1·00	1·00
122	1·20	1·63	1·34
123·30	1·67	2·82	1·65
125	2·69	5·14	1·91
127	5·04	12·44	2·47
128·30	13·21	55·82	4·23
130	50·02	225·72	4·51

Between 25 and 60 minutes I ascertained that striking the forehead smartly with the hand when the bands were just bright enough to show colour caused them to appear colourless for a few seconds, but on repeating the experiment at 105 minutes this effect was no longer produced.

This result is, I consider, of great interest. For if a pair of differently coloured bands, considerably brighter than the minimum visible, appear for a few moments colourless when the after-effects of previous illumination, or dazzle-tints, receive a transient intensification, the inference is plain that the colours of the minimum visible must be far more liable to be masked even without such intensification, and that no observation as to the impossibility of distinguishing colours in the minimum visible can be valid unless it has been ascertained that the dazzle-tints have completely subsided.

Relation of the Violet-blue : Red Ratio for the Minimum Visible by Daylight, to that for the Minimum Visible after Two Hours in Total Darkness.

In making these measurements I grasped the eye-piece of the spectroscope with my left hand so as to exclude all extraneous light from the eye. The sensitiveness of the eye changes so rapidly that it is necessary to have some time limit within which the bands must be perceived. The normal limit here adopted was one minute. If they were glimpsed before 55 seconds or after 65 seconds, the experiment was rejected.

Experiment 9.—Sitting with my back to the window on a dull December day with all blinds down but one, the violet-blue : red ratio was 70.

After looking out of the window for a minute or so it was 341, and on looking at the sky, which was very dull and cloudy for 10 seconds, it rose to 418. Ten minutes' rest with my back to the window brought it to 96. After writing for about 5 minutes on white paper it was 185. Ten minutes spent talking in an upstairs room brought it to 364, and after another 10 minutes with my back to the light it was 111.

Experiment 10.—On another occasion I took a series of measurements at half-hour intervals, working in another room in the intervals. The first measurement was taken directly after setting up the apparatus, and the second after going about the laboratory collecting materials for my other work. In the former case the influence of the subdued light of the room in the basement in which the apparatus was situated is evidenced by the lower reading and in the latter the effect of the more brightly illuminated rooms is very marked. The last three, when the intervals were spent under fairly constant conditions, agree tolerably well.

Each measurement was made independently, the Nicol being turned back

Minimum Visible by Daylight, in Terms of the Minimum Visible after Two Hours in Total Darkness.

Red.	Blue-violet.	Ratio.	
18·9	1060·4	56	After setting up the apparatus.
8·0	1070·0	134	After going about the laboratory.
14·4	1064·3	74	After working in an adjoining room.
14·0	1064·7	76	,, ,, ,,
13·1	1065·5	81	,, ,, ,,

through an unknown angle after the record was pricked. At the end of the series of experiments the angles corresponding to all the records were read off. As it is important in all measurements depending on sensations rather than physical quantities to avoid mental bias, I did not, until these experiments were completed, refer to my notes taken in 1901 of the literature of the subject. It is the more interesting to find that, in Charpentier's case, the eye increased in sensitiveness to white light at least a thousand times after a prolonged stay in darkness, and on one occasion two thousand five hundred times.

It should be observed that, in my experiments, no brighter light was used than that of a very dull and cloudy December sky, and that only for a few seconds. Judging from other experiments, I consider that Charpentier's highest figures must be well within the mark for a person coming from a fairly well-lit room in the summer time.

But it would appear that by far the greater part of this increase of sensitiveness relates to the more refrangible rays. In my own experiments, the minimum visible for blue-violet varied in daylight from 1060 to 1070 times its value after two hours in darkness, whereas the range for the red is proportionally much greater, although the largest value is barely nineteen and the smallest eight times the minimum visible to the dark-adapted eye. This larger value was probably due to fatigue of the red sensation induced by the glow-lamp while adjusting the illumination.

Time-relations of the After-Images and Dazzle-Tints.

I have reserved the description of the subjective sensations experienced in the dark room during these and other experiments, in order to discuss them separately. The notes of these observations were written at the time, in the dark, with pencil, in a small reporter's note-book. A fresh page was turned over for every fresh note, an indiarubber band round those written on preventing the same page from being used twice. An account of each experiment was written in the laboratory book, while the exact significance of the notes was fresh in my mind.

Time was recorded as follows:—A shaft carrying a grooved wheel 10 cm. in circumference was connected with the arbor of the hour-hand of a common Ansonia clock, so as to revolve with it. On a thread wound round this wheel, and attached to it, hung a small weight, to the bottom of which was soldered a toothed wheel of rather larger diameter than the weight.

The whole was mounted on the top of an upright case about 18 inches high, inside which the weight hung. Before making an experiment, a strip of white blotting-paper was secured to the back of the case with drawing-pins, the weight wound up to the top by turning the hour-hand backwards, and a mark made on the blotting-paper by pressing the toothed edge of the weight against it. This mark was entered in the note-book as the zero of time.

While in the dark room, before making each note, the weight was pressed against the blotting-paper so as to mark it. Immediately after the conclusion of the experiment, the weight was wound up to each mark in succession, and the corresponding time read off on the clock face. There is no difficulty in reading to 30 seconds, and there is ample space for a 3 hours' record. Except in a vague way, the observer does not know how the time is going on. If it were desirable to keep him in entire ignorance of it, an electrical time-marker might be used to record on smoked paper. I used this clock during each of the above-described experiments, and during many others made solely for the purpose of investigating the time-relations of these subjective phenomena.

The phenomena succeed one another in a well-marked order, but at no fixed time. As in the case of the duration of artificial colour-blindness,* and also of the rate at which flickering ceases for the various colours in experiments by intermittent light,† not only the condition of the eye as regards previous fatigue, but also the state of the health seems to exert a considerable influence on the time required for the eye to become completely rested. There are, however, very noticeable time-relations between the various stages of the process which preserve a certain proportionality among themselves.

Actual after-images—the ocular spectra of Newton—do not as a rule last more than 10 minutes or a quarter of an hour, unless they have been excited by looking for some time at a strong light, in which case they may come up again and again in the most unexpected manner, after remaining invisible for half an hour or more. On one occasion, on my way to the laboratory, I had for some minutes watched a bird nest-building in a tree. After turning out the gas in the dark room I saw after-images of the flame for about 10 minutes. These died out, and for some time the room seemed full of a luminous fog. Then gradually an image of the branches round the birds' nest developed, and persisted with more or less distinctness for nearly three-quarters of an hour. Under such circumstances, it is seldom worth while continuing the experiment, as the concluding stage is likely to be correspondingly delayed. It is better to avoid looking at the sky or any bright light for some hours before entering the dark room.

---

* 'Phil. Trans.,' B, vol. 191, 1899, p. 1.

† 'Journal of Physiology,' vol. 21, 1897, p. 426.

If this precaution is taken, the average course of events is fairly shown by the following figures taken from seven experiments, in which the final stage was reached in two hours. The times given are the earliest and latest at which the phenomena described commenced in any of the seven experiments :—

0 to 5 minutes. Confused after-images of portions of objects recently looked at. These images, with the exception, perhaps, of that of the gas or lamp flame, do not usually appear for the first 20 or 30 seconds after the light is turned out. They have hard outlines, and they gradually fade and give place to a luminous fog, made up of what I have called "dazzle-tints," *i.e.*, coloured impressions of luminosity without form.

10 to 15 minutes. Fog no longer uniform, but "spotty" with patches of a brownish or greenish-bronze colour.

18 to 21 minutes. A group of yellow or green luminous points—10 to 15 of them, arranged quincunx fashion, floats about, following the direction of the eyes. This phenomenon is, I believe, associated in some way with the yellow spot. It occurred in five out of the seven cases, lasting from 10 minutes to half-an-hour.

23 to 28 minutes. Green "dazzle-tints" predominate, beginning to break up, in some cases, into patches of green showing a fainter blue between.

40 minutes. Blue increasing—green weakening and breaking up into isolated patches.

66 to 72 minutes. The luminous fog, which now shows no trace of green, begins to break up into blue patches, with occasional black spaces between.

Generally after this, for a while it seems as if all the after-effects had gone. But on making a great effort of attention it becomes evident that they are still there.

Swinging the arms, or going through dumb-bell exercises will bring them back for a time.

106 to 120 minutes. Gradually a luminous violet fog seems to fill the surrounding space. For a minute or two it increases in brightness, and then breaks up near the middle in two or three places and seems to roll away on all sides.

Then it returns, and again breaks up, generally leaving an island about the size of the yellow spot, which lasts two or three seconds longer than the rest. The colour of these clouds is a rich pure violet like that of the calcium lines H and K in the arc spectrum. This phenomenon may go on from 10 minutes to half-an-hour, the violet patches getting smaller and appearing at longer intervals till they die away. When that has happened no more "dazzle-tints" appear, and in my own case *there is no longer any interval whatever* "*between the absolute and chromatic thresholds.*"

In other words, green is distinct from blue, and blue from violet, and violet from red as soon as either is strong enough to produce any sensation at all.

I desire to emphasize this conclusion. It is absolutely opposed to the statements of Hering,[*] Hillebrand,[†] Aubert, Charpentier,[‡] Landolt,[§] Vogel[||] and others, whose experiments have been regarded as classical. But it is the result of experiments made in the first instance with a mental bias in favour of the view I am opposing, and finally repeated with the improved appliances now at my disposal.

The times given above represent the normal duration of the various stages in the fading out of the after-effects of light upon the eye.

They are seldom shorter, but occasionally much protracted.

Sometimes the red "dazzle-tints" return after more than half-an-hour, and the green persist correspondingly longer. In 1895 several times I waited for nearly three hours without getting beyond the green. In January of the present year, on one occasion the green "dazzle-tint" persisted for 75 minutes instead of being quite gone at 40 minutes. I therefore discontinued the experiment because it would probably have involved waiting about four hours for the violet to pass off.

After-effects last longer in summer than in winter, perhaps partly because of the greater brightness of the daylight and its longer duration. As a rule I find them more persistent during ill health, but on one occasion, after anæsthetisation of the eyeball by cocaine, I saw the violet clouds one hour after the bandage had been put on, *i.e.*, one-half the usual interval.

In view of this great variation in the time required for the subsidence of after-effects, it is quite conceivable that with some persons they may persist so long as to be practically never absent. And in such cases there would always be, as some observers have maintained, an "interval between the absolute and the chromatic images." When my eyes have not quite lost the "dazzle-tints" there is, to me also, such an interval. But when they are completely rested there is none.

---

[*] Hering, "Untersuchung eines total Farbenblinden," 'Pflüger's Archiv,' vol. 49, pp. 563 to 609.

[†] Hillebrand, "Specifische Helligkeit der Farben," 'Wien Akad. Sitzber.,' 1889, p. 70.

[‡] Charpentier, 'Thèse de Doctorat,' 1877; also 'La Lumière et les Couleurs.'

[§] Landolt, 'Comptes Rendus,' vol. 86, p. 495, 1878.

[||] Vogel, 'Annalen d. Physik u. Chemie,' 1895, vol. 54, p. 745.

Experiment 11.—According to the theory of Young and of Helmholtz, each portion of the spectrum with the exception of its two ends, excites more than one colour sensation. My own experiments on artificial colour blindness support this view. The observations recorded in the present paper show that the effect of rest in darkness is to increase the sensitiveness of the eye very much more to the highly refrangible than to the less refrangible rays. It follows that a colour like that of the sodium flame which, to a normal eye, excites the red and green sensations in almost equal proportions will, if the intensity is greatly reduced and the eye sufficiently rested, excite a larger proportion of green. This I have put to the test of experiment. The results are interesting as bearing on the precautions necessary in such cases. A Bunsen flame in which was a piece of glass tubing containing crystals of sodium thiosulphate was placed at such a distance from a paper screen outside the dark room, that its light, reflected on to a second piece of white paper inside the dark room in the place usually occupied by the spectroscope, was barely visible to the perfectly rested eye. *It appeared whitish-grey even when considerably brighter than the minimum visible.* The reason of this was evident when I removed the second paper reflector and examined the light from the first with the spectroscope. In addition to the sodium lines there was the spectrum of the Bunsen flame itself with its three bands in the green, the blue, and the violet. But in the spectroscope, where these other colours were separated from them, the *sodium lines appeared pale green when of the minimum visible intensity.*

The sensitiveness of the eye to blue increases so greatly after two hours in darkness that nothing but spectrum analysis can ensure its absence—and a very small trace may suffice to change orange to white. For the same reason we cannot take the green of the spectrum at E or *b* as the standard green for the minimum visible. We know that the red and the blue sensations overlap there, even if the violet does not reach so far. The colour must look pale under feeble illumination owing to the presence of three if not all of the constituents of white. The greatest contrasts seem to be obtained, so far as I have gone, with light from B for the red, from D for the green, from F for the blue, and from H and K for the violet.

Owing to this overlapping of the colours, I have not yet satisfactorily determined the increase in the sensitiveness of the rested eye to green. It appears to be less than the increase for blue.

There is no colour about which so much latitude of expression is used as about white. Being composed, according to Newton's view, of all the colours, it is more affected by variations in the composition of the light than, for instance, red, which may appear brighter or darker, but cannot change its hue. We habitually discount this effect in the case of white, without doing so for the other colours. Thus purple flowers often look red by lamp-light, and are spoken of as red; but white paper, though it looks yellow, is called white. And we do so whether using candles, arc lights, or incandescent gas, though the actual hue of the paper is different with each, and is strikingly so when they are compared side by side with daylight. With regard to the colour or colourlessness of very feebly illuminated objects, no experiment should be considered valid in which the colours are not contrasted. None but spectral colours should be used, and even these, owing to the overlapping of the colour sensations, must of necessity appear pale. Few persons have the faculty, which dyers by long practice develop, of being able to match colours by memory. Yet this is what has to be done in order to say, after two hours in absolute darkness, whether a colour so faint as to be barely visible is pale yellow, pale green, or pale blue.

To sum up:—In my paper on Artificial Colour-Blindness, I described experiments showing that Hering's argument in favour of a black-white sensation is invalid, in so far as it rests on the statement that by intense light all colours tend towards white. For the apparent whiteness—in the green region, for instance—is only a transitory stage in the production of green blindness, and is reached when the green sensation is reduced to the strength of the underlying blue and red, the mixture of the three being equivalent to white by candlelight, and, therefore, by courtesy, white. And if we continue the experiment the whiteness gives place, as the eye becomes completely green-blind, to rich red and blue.

I submit that the experiments described in the present paper indicate that Hering's theory of the black-white sensation is also invalid in so far as it rests on the statement that by very faint light all colours appear white.

For in this case also my experiments, many times repeated and extending over a number of years, show that the apparent whiteness is only a transitory stage in the recovery from the after-effects of light, and is due largely to positive as well as negative after-effects. And when the "dazzle-tints," as I have called them, have completely subsided, there is no interval between the threshold of light-sensation and the threshold of colour-sensation.

With the exception of the earlier experiments this research has been conducted partly at University College, Reading, and partly at the Physiological Laboratory, Oxford. The expense of some of the experiments has been defrayed from the Government grant.

My thanks are due to Mr. W. W. Fisher for the loan of apparatus from the Marlborough Collection, and to Professor Gotch for the facilities afforded in his laboratory. And I desire to acknowledge the assistance rendered during my stay in the dark room by the laboratory attendant.

On the Nature of the Silver Reaction in Animal and Vegetable Tissues.

By A. B. Macallum, M.A., M.B., Ph.D., Professor of Physiology in the University of Toronto.

(Communicated by W. D. Halliburton, F.R.S. Received March 3,—Read April 6, 1905.)

I. *Introduction.*

Though the use of nitrate of silver as a reagent in histology has now a half century of history, and has been of the greatest service in elucidating the structure of some tissues, it has not yet been determined how the reagent is affected by the tissues, or what compound or compounds in the tissues are responsible for the precipitate which becomes discoloured in the sunlight. There are, indeed, references to these points in the literature of the subject, but these are scanty, and they are not at all in accord, while the majority are only guesses or fanciful explanations of the reaction itself. It may even be said that the investigators who used the reagent directed the whole of their energies to determining what it showed from the morphological side rather than what was involved in the reaction itself, and because of this there has been nearly 30 years of discussion on the question whether the results obtained with the reagent were trustworthy or were of the nature of artifacts.

The principal value of the reagent to the earlier observers appeared to be the fact that, after impregnation with nitrate of silver, and exposure of the preparation to light, cell outlines were revealed, and thereby the structure of lymphatics and of lymph tissue were demonstrated, the outlines being shown through the brown reaction which they manifested in such preparations. Von Recklinghausen,[*] who was amongst the first to use the reagent for this purpose, held that the silver salt is deposited in what he regards as the cement substance between the cells, and that this cement material under the influence of light reduces the silver. It was pointed that the reaction is not confined to the cell peripheries and intercellular material, for preparations sometimes showed the reaction present in the cytoplasm of the cells, but absent from the membranes and the intercellular structures. This condition was known as the positive reaction or positive image, and was attributed by Schweigger-Seidel[†] to the decomposition and redistribution of the silver precipitate which, in the normal cell giving the negative image, is to be found in the intercellular spaces and boundaries, that is, in the cement substance of Von Recklinghausen. Schweigger-Seidel maintained that the decomposition was brought about by chlorides forming silver chloride from the silver albumen precipitate in the intercellar material, the silver chloride so formed dissolving in the presence of chlorides, and thus diffusing into the cytoplasm, where, under the influence of light, it became the subchloride, and he proved this by taking preparations which had been treated with nitrate of silver, but not acted on by light, and placing them away from the light in a solution of sodium chloride. In these, when reduced by the light, the coloured silver salt or compound obtained chiefly in the cytoplasm. According to Hüter* the positive image may arise through diffusion of the cement substance into the cytoplasm and the consequent intracellular precipitation of the silver compound. His,† in 1862, held that the silver compound in the cornea which reduces in light is not an albumin compound, but chloride of silver, for when the cornea was treated with mercuric nitrate, which dissolves chloride of silver, all the silver precipitate was dissolved, which would not have been the case had any of the compound been silver albuminate. In later observations,‡ however, he admits that the silver compounds may be an albuminate as well as a chloride, and that both reduce under the influence of light. A similar view was held by Harpeck,§ Hartmann,|| Auerbach,¶ and Henle.** Schwalbe†† found that if the serous membrane to be examined be first washed with a 4-per-cent. sugar solution, treatment with silver nitrate will not bring out the silver lines usually obtained, and he concludes from this that the cement substance, such as Von Recklinghausen postulates, has nothing to do

---

[*] 'Die Lymphgefässe und ihre Beziehung zum Bindegewebe,' Berlin, 1862.

[†] "Ueber die Grundsubstanz und die Zellen der Hornhaut des Auges," 'Berichte d. Kön. Sächs. Gesell. d. Wiss., Math.-Phys. Cl.,' vol. 20, p. 305, 1868.

---

* "Zur Pathologie der Gelenkflächen und Gelenkkapseln, mit einem Kritischen Vorwort über die Versilberungsmethode," 'Arch. für Path. Anat. und Physiol.,' vol. 36, p. 25, 1866.

† "Ueber die Einwirkung des Salpetersauren Silberoxyds auf die Hornhaut," 'Schweizer Zeitschr. f. Heilkunde,' vol. 2, p. 1, 1862.

‡ "Ueber das Epithel der Lymphgefässwurzeln und über die Von Recklinghausen'schen Saftcanälchen," 'Zeit. f. wiss. Zool.,' vol. 13, p. 455, 1863.

§ "Ueber die Bedeutung der nach Silberimprägnation auftretenden weissen lücken- und spaltähnlichen Figuren in der Cornea," 'Arch. f. Anat.,' 1864, p. 222.

|| "Ueber die durch den Gebrauch der Höllensteinlösung Künstlich dargestellten Lymphgefässanhänge, Saftcanälchen und epithelähnlichen Bildungen," 'Arch. f. Anat.,' 1864, p. 235.

¶ "Untersuchungen über Blut- und Lymphgefässe," 'Arch. f. path. Anat. und Phys.,' vol. 33, p. 340, 1865.

** "Bericht über die Fortschritte der Anatomie im Jahre 1866," 'Zeit. f. rat. Med.,' 3e Reihe, vol. 30, p. 6.

†† "Untersuchungen über die Lymphbahnen des Auges und ihre Begrenzungen," 'Arch. für Mikr. Anat.,' vol. 6, p. 1, 1869.

with the reaction, that the latter is due to an albuminous layer, "an albuminous cement substance," adhering to the edges of the cells, and which can be washed away.

Legros* was of the view that in the treatment with the reagent the whole cell received a slight stain, and that the dark lines were the contact surfaces of two feebly stained cells. Feltz† held that all the silver lines are artificial products, basing this view largely on the fact that in membranes made from albumin and collodion, similar reticula may be obtained by treatment with silver, and like figures were produced by Severin‡ on surfaces which contained no epithelium. Soboroff§ accepted Von Recklinghausen's view which, however, Reich|| rejected, holding that the silver lines do not originate from cement substance, but in part from a fluid between the cells, but what the nature of the precipitate is he did not decide. Robinski¶ also does not admit the existence of a cement material, and he found that the cell borders, in *e.g.*, the epithelium of Descemet's membrane, colour first, then the reaction slowly advances into the cytoplasm, and finally the whole cell is stained, which ought not to be the case if Von Recklinghausen's views are correct. A cement substance is unnecessary to bring about contact and adhesion of cells to each other. According to Alferow** the silver precipitate, which colours under the influence of light, is a mixture of the chloride and the albuminate, as the free acids (picric, acetic, lactic, and citric) which he used dissolve all the other precipitates. Adamkiewicz,†† on the other hand, held that the silver lines are albuminate of silver only, and that they are laid down in a cement substance.

The reagent was also employed by Ranvier very much in his histological researches, but he has ventured no explanation of its action.

---

* "Note sur l'épithélium des vaisseaux sanguins," 'Jour. de l'Anat. et de la Phys.,' 1868 p. 275.

† "Recherches expérimentales sur le passage des leucocytes à travers les parois vasculaires," 'Jour. de l'Anat. et de la Phys.,' 1870, p. 33.

‡ "Beiträge zu der Lehre von Entzündungen," 'Diss.,' Dorpat, 1871.

§ "Untersuchungen über den Bau normaler und ectatischer Venen," 'Arch. für path. Anat. und Physiol.,' vol. 54, p. 137, 1871.

|| "Einige mikroskopische Studien mit Silbersalpeterlösung besonders an Gefässen des Auges und anderer Organe," 'Sitzber. d. Wiener Akad.,' 1873, vol. 67, p. 81.

¶ "Recherches microscopiques sur l'épithèle et sur les vaisseaux lymphatiques capillaires," 'Arch. d. Physiol.,' 1869, p. 451; also "Die Kittsubstanz auf Reaction des Argent. nitric.," 'Arch. f. Anat.,' 1871, p. 184.

** "Nouveaux procédés pour les imprégnations à l'argent," 'Arch. de Physiol.,' 1874, p. 694.

†† "Ueber die Behandlung von Gefässen mit Silbernitratlösung," 'Berl. Klin. Woch.,' 1874, p. 355.

Amongst the more recent observers, Boveri* holds that the material in which the silver precipitate occurs is not a specific substance, either cement or otherwise, but only the contact surfaces of two neighbouring cells. He claims that he observed, inside of blood vessels, red blood corpuscles lying in contact with each other, and between the contact surfaces the silver precipitate occurred as between endothelial cells. What the precipitate itself is he does not say. Rabl† regarded it as probable that the reagent combines with the albumin to form a silver-nitrate proteïd, through union of the molecules, analogous to that process which operates in the precipitation of urea with mercuric salts. That the brown granules of the precipitate are not metallic silver is shown by their solubility in sodium thiosulphate. According to Mann‡ the precipitate is probably a proteïd in combination with chlorides and carbonates.

From this review it will be seen that there is as yet much uncertainty as to the nature of the reaction which silver nitrate undergoes in tissues under the influence of light, some observers holding that the silver compound is a mixture of a chloride and an albuminate, both of which become coloured when exposed to the light, while others postulate the presence of an albuminate compound only.

The cause of this confusion lies in the fact that our knowledge of the action of organic compounds on silver salts under the influence of light is very indefinite and fragmentary. It is, of course, known that when a silver salt is added to a solution of one of certain organic compounds, and the mixture exposed to light, a more or less coloured product soon appears, the formation of which is supposed to be due to reduction. This is a term which applied to a salt of silver, has a wide meaning, one application comprehending that decomposition of the salt in which metallic silver is set free, another involving that change of the salt in which the quantity of the element or substance combined with the silver is diminished. Both of these types of reduction are illustrated in the case of a salt of silver in association with organic compounds.

The reduction to the metallic condition occurs when alkaline silver solutions come in contact with living protoplasm (Loew and Bokorny),§ and it occurs also when certain organic compounds in alkaline solution—*e.g.*, uric acid, levulose, dextrose, hydroxylated benzol derivatives, hydrazine and aldehyde compounds—are heated with nitrate of silver. In neutral solutions the hydroxylated benzol compounds have the same effect on silver salts. In these cases the metallic silver is black, and it is not soluble in the solutions (sodium thiosulphate, etc.), which dissolve argentic or argentous chloride.

---

* "Beiträge zur Kenntniss der Nervenfasern," 'Abhandl. d. Kön. Bayer. Akad., Math.-Phys. Cl.,' vol. 15, p. 421, 1886.

† "Ueber geschichtete Niederschläge bei Behandlung der Gewebe mit Argentum nitricum," 'Sitzber. Wiener Akad., Math.-Phys. Cl.,' vol. 102, Abth. 3, p. 342, 1893.

‡ 'Physiological Histology, Methods and Theory,' Oxford, 1902, p. 266.

§ 'Chemische Ursache des Lebens.,' München, 1881.

The reduction involving diminution in the quantity of the element combined with the silver is, of course, well known in the case of the haloid salts, the chloride AgCl, for example, becoming converted by the action of light into the subchloride Ag_2Cl, which is violet, reddish-violet, blueish-violet in mass, but it may also be reddish-brown when occurring in thin layers, membranes, or deposits, the shade of colour apparently depending on the presence of the subhaloid in a finely divided form or otherwise. The quantity so converted is, according to Carey Lea,* not more than 1 per cent. of the total haloid salt, with a portion of which it combines, the compound formed not containing more than 9 per cent. of the subchloride. Hodgkinson,† however, regards the salt formed by the action of light on silver chloride as an oxychloride, probably of the composition represented by the formula Ag_4OCl_2, in which case there is no reduction, the change merely involving a substitution of oxygen for half the chlorine. There is no doubt about the loss of chlorine, as simple experiments indicate such a loss, but whether the coloured salt is an oxychloride or a simple subchloride does not matter for the present, although Carey Lea is pronounced in the view that the compound is a simple subhaloid, while Guntz‡ was able to prepare the subfluoride of silver Ag_2F, from which, through the action of chloride of carbon, of silicon, and of phosphorus he obtained Ag_2Cl. He further obtained by the same method the subiodide Ag_2I, the subsulphide Ag_4S, and the suboxide Ag_2O.

There is also the difficulty presented by the proteïds. The current view held, not only by the chemists, but also by scientific exponents of photography, is that proteïds form coloured reduction products with silver, and that organic matter generally gives similar reduced compounds. Upon this point the evidence has appeared decisive, for if egg "albumen" or serum "albumen" be treated with acid nitrate of silver the result is a precipitate which in the sunlight quickly becomes coloured. Further, if gelatine in solution be similarly treated, a precipitate may not occur, but the colour reaction quickly appears, and is usually of a pronounced character. This summarises the evidence, and, taken in conjunction with the fact that certain organic compounds in neutral or alkaline solution, in sunlight, "reduce" salts of silver, seemed to indicate very distinctly that the coloured silver compound produced in solutions of proteïds is due to the latter, it being supposed that an "albumenate" of silver obtains which in light forms a compound, argentous "albumenate," analogous to the argentous haloid salt of the photographic plate.*

---

* "On some Reactions of Silver Chloride and Bromide," 'Amer. Jour. of Science,' 3rd series, vol. 15, 1878, p. 189; also "On Red and Purple Chloride, Bromide and Iodide of Silver, on Heliochromy and on the Latent Photographic Image," 'Amer. Jour. of Science,' vol. 33, p. 349, 1887.

† Meldola, 'The Chemistry of Photography,' 1889, p. 56.

‡ 'Comptes Rendus,' vol. 112, pp. 861 and 1212.

All these facts make it possible to understand how it came about that there was any discussion as to the nature of the silver reaction in tissues, and why it was accepted without much question that proteïds took a part in this reaction. The author has thought the subject worthy of further investigation, on the ground that if the silver reaction is due to the presence of few or many organic compounds, as well as to the presence of haloids, the accurate localisation of chlorine, bromine, and iodine in tissues is possible only under great difficulties, whereas if the reaction is due to haloid salts, or can be applied so as to demonstrate the presence of haloid salts only, the cyto-chemist has a means of determining the presence of not only chlorine, bromine, and iodine, but also to a certain extent of sodium and potassium in tissues.

It has for several years been the view of the author that the reaction of proteïds with nitrate of silver in sunlight is due to the presence of chlorides only, and that if proteïds could be thoroughly freed from chlorides, the former would give no reduction compound with the silver salt. The demonstration of this, when attempted, was beset with difficulties, due partly to the fact that chlorides, and particularly the chloride of sodium, are present everywhere, and, therefore, contaminating, as they do more or less, every reagent and preparation, the absolute removal of chlorides appeared to be impossible of accomplishment, and in part, also, to the extreme sensitiveness of the reaction which demonstrates the presence of the haloid salts. Silver nitrate will demonstrate the presence of one part of chlorine in 1,000,000 parts of water, but I have found, also, that if sunlight is allowed to act on the preparation, one part of sodium chloride may be detected in 1,000,000 parts of water—that is, 1 part of chlorine in 1,600,000. It is manifest that this test is exceedingly delicate, and consequently the reaction, if properly sought for, could be obtained in all fluids, and particularly with colloids, which are very tenacious of the inorganic salts with which they are associated. Because of this, it appeared hopeless to attempt to free proteïds from haloids, and thus demonstrate the inactivity of proteïds towards salts of silver.

The results of researches recently carried out regarding the detection and localisation of potassium salts in animal and vegetable tissues, having indicated how important it is to determine whether anything else than halogens affect the silver salt in light, I was led to take up the question anew on lines that would promise a definite solution of the problem. The results of these observations seem to be decisive, and therefore these and the methods by which they were reached may now be described.

---

* Meldola, *op. cit.*, pp. 116 to 119 and 342 to 352.

II. *Methods and Results.*

There are, beside the haloid salts of silver, other compounds of the same metal which undergo in the presence of light a change which is termed reduction. Some of these are important, but only those were examined which appeared likely to affect directly the question of the nature of the silver reaction in tissues.

The phosphate when precipitated darkens on exposure to light, but if free nitric acid is present, the precipitate does not occur and the dark reaction fails to appear. The carbonate also darkens, but in the presence of nitric acid the nitrate is formed, and this does not undergo reduction. The sulphate is unaffected, but the sulphocyanide is "reduced," undergoing a slight change in colour, even in the presence of nitric acid. The hippurate, the oxalate, the valerate, the palmitate, the oleate and the stearate are unaffected, while the glycero-phosphate acts like the phosphate in the presence of nitric acid, and lecithin also does not affect the silver salt. The tartrates and citrates in the presence of nitric acid are unaffected. Further, the acetate, the amido-acetate, the amido-propionate, the succinate and the lactate, when pure, give no reaction in the same acid medium. Of the more strictly physiological compounds, taurine and creatine act on the acid solution of the silver salt, producing in the light a coloured reduction compound, and cyanuric acid acts similarly, yet less readily, while alloxan and alloxantin immediately reduce the silver to the metallic condition, but purins, urea, leucine, tyrosine, indol, skatol, and their derivatives exercise no effect.

This makes it certain that only a few compounds of the extractive class may affect the silver reaction in tissues, and, with the exception of creatine, their presence may be disregarded, for they are excessively small in amount when occurring at all in tissues. Creatine, on the other hand, is abundant in the striated muscle of vertebrates, while it is absent wholly from invertebrates. One may consequently avoid the difficulties presented by the occurrence of creatine simply by using for investigation the muscle tissue of invertebrates.

That part of the result of the reaction in tissues is due to haloid salts of silver formed there seems to be beyond doubt. Sodium and potassium chlorides are constituents of all tissues, animal and vegetable, and it is possible to extract them with water free from haloids, and thereby greatly affect the silver reaction of the tissues so treated. In the case of vegetable stems also, the salts may be extracted with alcohol of 98-per-cent. strength, which, of course, leaves in the preparations their proteïds and carbohydrates and sections made from vegetable tissues (*e.g.*, *Tulipa*), and at once treated with alcohol for 24 hours, usually give no reaction with the silver reagent. This is true also of animal tissues, thin sections of which, made from fresh material frozen with carbon dioxide spray, after lying in alcohol of 90 per cent. for some days, are practically unaffected in a silver solution placed in the sunlight. Stronger alcohol, even of 98-per-cent. concentration, also removes the chlorides if the pieces of tissue be small or in thin sections, but if the pieces be of considerable thickness, the alcohol removes only a small portion of the salts and redistributes the remainder throughout the preparations.

The question is, however, not whether the haloids in tissues constitute a factor in the silver reaction, but rather whether apart from the exceptions referred to they are the only compounds which contribute to the reaction.

The proteïds, as already pointed out, are regarded as possessing the property of forming reducible compounds with silver, and to determine whether this view is correct, the purification of a number of typical proteïds and of an albuminoid was undertaken.

For this purpose it was necessary, first of all, to have every fluid and reagent that was used free from chlorides to the extent that these were below the minimum limit of detection, that is, if chlorine should be present as chloride it would be less than 1 in 1,600,000. This is possible in the case of carefully distilled water, but it is not quite as easy in the case of the precipitating reagents employed, namely, anhydrous sodium sulphate* and ammonium sulphate. In the case of the former, the "chemically pure" material had to be repeatedly crystallised before it was obtained in a form sufficiently free from chlorides. Many of the "chemically pure" preparations of ammonium sulphate put on the market are not of the standard of purity required, and different quantities of the salt from the same manufacturer were found to vary considerably as regards relative purity. In every case, however, only those preparations of the salt were used which reached the standard exacted.

The material employed consisted of egg "albumen," serum "albumen," and gelatin. In the case of egg "albumen" the solution was first of all made in a large quantity of distilled water with the consequent separation of globulin, from which, on sedimentation, the supernatant solution was removed by decantation, and from this the albumins were precipitated on the addition of enough ammonium sulphate to saturate the solution. Tested with silver nitrate solution,* a precipitate was obtained which gave a deep reaction in light in less than 20 minutes. The precipitate obtained with the sulphate was dissolved and again precipitated in the same way, the precipitate again dissolved and re-precipitated, this process being repeated in one case 9 times, in another 14 times, and in a third 11 times before the desired degree of purity was attained. It was subsequently found that if in each case when the proteïd precipitate is re-dissolved the solution be allowed to stand for some time, for example, 10 to 12 hours, before the subsequent precipitation is brought about, the standard of purity may be reached before the eighth precipitation occurs. It would seem as if the chlorides are in intimate union with the particles of the colloid, and that when precipitation immediately follows solution, salts are largely retained in the interior of the colloid molecule groups, but when the latter are acted on for hours by water free from chlorides, the latter pass into the water, with the result that when precipitation occurs much less of the chlorides is carried down. The effect of this may be seen when the filtrates are tested for chlorides. When the precipitations followed each other at very short intervals the chloride reactions of the filtrates were slight, but when the precipitated proteïd was dissolved and allowed to remain in solution in each case some hours before being again precipitated, the filtrate gave a much deeper reaction and of the typically chloride character. When the silver precipitate from the earlier filtrates was allowed to settle, the supernatant fluid decanted, the precipitate then washed several times in water free from haloids, finally collected in a porcelain crucible, dried at 120° C. for three to five hours, weighed, then fused and once more weighed, there was found to be practically no loss of weight, this fact showing that organic compounds do not enter into the composition of the silver precipitate.

---

* On the employment of anhydrous sodium sulphate as a precipitant of proteïds from their solutions, see Pinkus: "On the Precipitation of Proteïds," 'Jour. of Physiol.,' vol. 27, p. 57, 1901 to 1902.

The albumins and globulins of serum were not separated from each other, it having been found easier to carry out one series of precipitations for both classes of proteïds in order to free them from chlorides. As a rule, it required more precipitations to purify the proteïds of serum than was the case with egg albumins. What the reason for this difference is is not clear.

The globulins which separated when egg "albumen" was dissolved in distilled water were freed from chlorides only by extraction with distilled water, and this was continued for two weeks or more, the water being frequently removed by decantation and as often replaced by fresh fluid. Portions of the globulin thus undergoing extraction were from day to day dissolved in dilute solutions of ammonium sulphate, free from chlorides, and, after the addition of some of the silver reagent, placed in the sunlight for days to determine how far the purification had advanced.

---

* The solution of silver nitrate used was exactly decinormal, and it contained 25 c.c. of nitric acid (60 per cent. strength) to the litre.

It is important not only in the case of globulins, but also in that of all albumins to have them in solution when the reagent is to be added. In the precipitate which silver nitrate produces, the latter is intimately mixed throughout with the proteïd. This is an advantage, for when the reagent is added to the undissolved proteïd it penetrates very slowly, and, consequently, the superficial reaction, if any occurs, may be so slight as to escape observation.

The egg albumins and the serum albumins and globulins which had undergone the indicated number of precipitations as well as the egg globulins which had been extracted for over a fortnight with distilled water, did not yield any reaction whatever with the silver nitrate reagent, even after weeks of exposure to bright sunlight, although the original unpurified material in every case gave an intense reduction effect.*

The purification of gelatin is more easily attained. For this purpose one dissolves the gelatin in water at 45° C. and adding to the solution, while it is maintained at that temperature, anhydrous sodium sulphate till the mixture is saturated. Stirred with a glass rod, the greater part of the gelatin collects on it and it can be thus lifted out of the solution and transferred to, and dissolved in, a fresh quantity of distilled water at 45° C., to which also anhydrous sodium sulphate is added till saturation obtains. The precipitated gelatin is once more in the same way transferred to a fresh quantity of distilled water at 45° C. The process of precipitation and solution was repeated many times, but each stage required only a few minutes, and consequently as many as twelve precipitations and as many solutions of the gelatin were obtained in two and a half hours. The purified product was found to set firmly and was in every case very clear and transparent.

The gelatin of commerce gives an intense reaction with the silver reagent in sunlight, but the gelatin of the ninth precipitation gave not the slightest reaction with silver nitrate solution after two weeks in sunlight, not even producing a precipitate or an opalescence. It mattered not from what crude preparation of commerce the purified product was obtained, the result was in every case the same.

That the compound or compounds in crude gelatin which react with silver nitrate in the sunlight are chlorides only, was demonstrated satisfactorily.

---

* When a neutral, instead of the acid, solution of nitrate of silver was used the result was the same. This is true also of purified gelatin similarly treated.

For this purpose the process of purification was modified slightly, the gelatin precipitated from the warm solutions was separated by filtration in a hot funnel, the filtrate cooled down to 5° C., with the consequent crystallisation of the greater part of the sodium sulphate. The mother liquor was then concentrated by evaporation, carefully filtered, and on cooling again another quantity of crystal sodium sulphate formed.* The mother liquor of this crystallisation gave, on the addition of a quantity of the silver reagent, a precipitate which, after being kept in a porcelain crucible at 120° C. for five hours, was of the same weight as it gave when it was fused. This could only be a haloid salt of silver and, therefore, organic compounds of silver do not obtain in the precipitate. The filtrates from the first, third, and fifth precipitations of gelatin gave such silver haloid precipitates, but in quantities diminishing in the order named.

Attempts were made to prepare nucleo-proteïds in a pure form, but these were unsuccessful, simply because the one efficient precipitant of these compounds from their solutions is dilute hydrochloric acid and enough of this reagent always adhered to or was united with the precipitates to give a distinct silver chloride reaction. The same difficulty was experienced in the case of nucleic acid.

It was not necessary, however, to prepare purified nucleo-proteïds or nucleic acid, for when animal and vegetable tissues in fresh condition are treated with the silver reagent and then exposed to light, the nuclei, if normal, are never affected and, therefore, not only is haloid chlorine absent from nuclei, but also nucleo-proteïds do not react with the silver salt. Further, as the head of the male element in the frog and *Oniscus* gives no reaction with the reagent, it may be inferred that the simpler compounds of nucleic acid are also unaffected by nitrate of silver.

No attempt was made to isolate vegetable proteïds in a form free from chlorides, but that they also do not give coloured products when treated with nitrate of silver and placed in the sunlight, seems to be clear from simple experiments which can be readily made on vegetable tissues. When thin sections of any succulent vegetable stem (*e.g.*, of *Tulipa*) are treated with the reagent, sunlight brings out a deep reaction in every part of the preparation, not only in the protoplasm, but also very frequently in the cell walls, the nuclei alone, when normal, never exhibiting the slightest reaction. When, however, the sections were first placed for a couple of hours in 99 per cent. alcohol, no colour reaction whatever developed on treatment with the silver reagent in sunlight. Alcohol of this strength* dissolves a considerable amount of sodium chloride and a smaller quantity of potassium chloride, and as the amount of each salt present in a section is small, the alcohol is capable of extracting it wholly, but leaving the proteïds at the points where they came in contact with the alcohol. That the complete absence of a silver reaction in sections so treated is due to removal from them of the salts, was shown when the alcohol which had covered for several hours a large number of sections was completely evaporated, the residue giving a very distinct reaction for chlorides.

---

* The filtration had to be done very carefully, in order to remove organic compounds which unite with silver nitrate, and which are thus precipitated, but such silver compounds do not discolour in sunlight.

When the preparations were covered for two hours with a mixture† of equal volumes of ether and acetone the greater part of chlorides were left in the sections, though not at the very points where they were during life, and such sections treated in sunlight with the silver reagent gave a marked colour reaction.‡ When the sections were carefully dried and then placed in the mixture for two hours their reaction was not perceptibly less marked than in fresh sections placed directly in the reagent. It was also found that sections which were first dried, then kept in a quantity of the acetone-ether mixture for 2 hours, and finally treated for 10 hours more with 99 per cent. alcohol, gave on the addition of the silver solution no colour reaction. The residue left on evaporation of the alcohol consisted of minute crystals of chlorides, apparently of sodium and potassium.

From all this it would appear that vegetable proteïds, like those of the animal kingdom, do not give with nitrate of silver dissolved in a dilute solution of nitric acid a colour reaction under the influence of light, and that the compounds in vegetable tissues which do react with the silver reagent are soluble in 99-per-cent. alcohol, but do not dissolve to any appreciable extent in the acetone-ether mixture. These facts do not exclude the possibility of the participation of other compounds than chlorides in the reaction which vegetable tissues give, but they seem to indicate that if there are such compounds they must occur in excessively minute amounts, and their presence is, when the silver reaction is obtained, masked by that of the chlorides.

---

* Sodium chloride is soluble in absolute alcohol to the extent of 65 parts in 100,000, and potassium chloride to the extent of 34 in 100,000 (De Bruyns, 'Zeit. für physik. Chem.,' vol. 10, p. 783). 99 per cent. alcohol takes up a much larger quantity of each salt.

† Such a mixture if made from pure anhydrous acetone and ether, according to the author's determinations, dissolves in 24 hours very little of the chlorides, 1,000,000 parts taking up only 2·4 parts of sodium chloride and 3·5 parts of potassium chloride.

‡ The acetone-ether mixture is a very rapid fixative for animal and vegetable tissues. Small pieces of kidney, liver, stomach, pancreas, thyroid and muscle when placed in it were rendered so firm in half an hour that very thin sections could be made from them with the section knife held in the free hand. The mixture is, on account of the rapidity of its action perhaps, not a serviceable histological reagent.

III. *General Remarks.*

The results given in the preceding pages make it quite clear that the reaction which animal and vegetable tissues give with nitrate of silver dissolved in dilute nitric acid may be attributed to halogens in haloid form,* and to taurine and creatine, and that proteïds and gelatin do not, when freed from traces of haloids, give the slightest colour reaction with the reagent. Of the two organic compounds which give the colour reaction, taurine may be neglected, since it obtains in infinitesimal quantities in animal tissues, and creatine, although present to the extent of 0·21 to 0·39 per cent. in frog's muscle, and of 0·4 per cent. in rabbit's muscle,† occurs in inappreciable quantities in other organs and it is absent altogether from invertebrate tissues.‡ One can, therefore, by appropriate selection of tissues of animal and vegetable forms for treatment with the reagent, determine, with a considerable amount of certainty and a very great degree of accuracy, the distribution of chlorides and, perhaps also, of other haloids, in various cytological elements.

This determination has already been made in the case of a number of cellular structures, and the results which have been obtained are of very great interest. These will form the subject of another paper, but two of them, which stand out in special prominence, are *that intercellular material and structures, including the so-called cement substance of Von Recklinghausen, are rich in chlorides, and that normal nuclei of animal and vegetable cells are absolutely free from them.*

---

* Compounds containing chlorine in a "masked" form as, for example, trichloracetic acid, give the silver haloid and subhaloid reactions after several days only.

† F. Nawrocki, "Ueber die quantitative Bestimmung des Kreatins in Muskeln," 'Zeit. für anal. Chem.,' vol. 4, p. 330, 1865.

‡ Krukenberg, 'Vergleichend-Physiologische Vorträge,' p. 316, Heidelberg, 1886; also Krukenberg's papers in 'Untersuchungen a. d. Physiol. Inst. d. Univ. Heidelberg,' vols. 3 and 4, 1880 and 1881.

On the Resemblances existing between the "Plimmer's Bodies" of Malignant Growths, and Certain Normal Constituents of Reproductive Cells of Animals.

By J. Bretland Farmer, F.R.S., J. E. S. Moore, and C. E. Walker.

(Received April 11,—Read May 11, 1905.)

It is proposed in the present communication to present the results of investigations bearing on the nature of those remarkable structures known as "Plimmer's Bodies."* As is well known, these are found in many cancerous growths, and are most commonly encountered in the younger or growing regions of the tumour. They appear in the form of vesicles, and they consist essentially of a fairly well-defined wall containing a clear space in which is suspended a small darkly staining granule (figs. 1, 2, and 3). They are most commonly to be met with in tumours of a glandular or glandular-epithelial origin.†

Fig. 3.

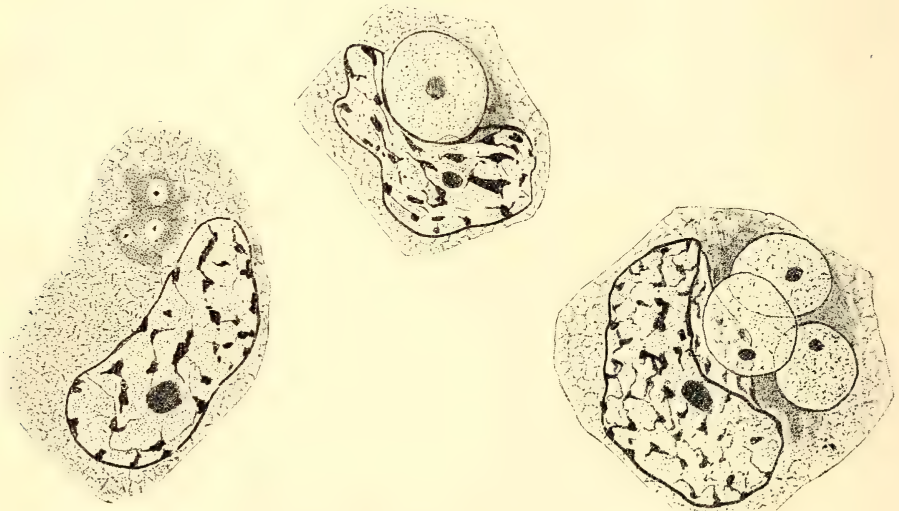


Fig. 1. Fig. 2.

Figs. 1, 2, 3. Examples of Plimmer's Bodies from carcinoma. 1. Three small "Bodies" in an archoplasm. 2 and 3. Later stages in the development of the "Bodies."

---

* Plimmer, 'Practitioner,' vol. 62.

† Greenough, '3rd Rep. Caroline Brewer Croft Cancer Com.,' Harvard Med. School, 1905.

They lie in the cytoplasm of the cancer cell, and usually in close proximity to the nucleus. In size, they vary from excessive minuteness to that of the nucleus itself.

The special interest attaching to them depends on the fact that they have commonly been regarded as peculiar to cancerous cells, although Honda* believes he has occasionally also encountered them in inflammatory tissues. They have been variously interpreted. Some investigators have regarded them as parasitic organisms, more or less intimately connected with the etiology of the disease, whilst others have seen in them a differentiation of the cytoplasm of the cancerous cell itself. It has been suggested also that they might be derived from the centrosomes within the archoplasm,† but the observations of Benda‡ that centrosomes coexisted independently of them in the cell, has rightly been held to disprove this hypothesis.

Our own investigations indicate, however, that there are good grounds for reconsidering the whole position, and a comparison of the processes that normally obtain during the final stages of development of the reproductive elements in man and the other mammalia, appear to us strongly to suggest that a parallel between the Plimmer Bodies of cancer and certain vesicular structures occurring regularly in the gametogenic, but not in the ordinary somatic, cells, may be found to hold good.

It was shown by one of us,§ in 1895, that during the prophase of the heterotype (first maiotic) mitosis of the spermatogenetic cells, the archoplasm undergoes a highly characteristic and peculiar metamorphosis. In normal somatic, or premaiotic, cells the archoplasm is seen to lie beside the nucleus as a dusky mass of protoplasm in which are contained the centrosomes. That is, the attraction sphere consists of the archoplasm *plus* the centrosomes.

But during the prophase of the heterotype mitosis these constituents become separated. The centrosomes are found to lie *outside of* and detached from the archoplasm (fig. 4). At the same time the archoplasm itself undergoes a change. It becomes vesiculated, and finally, at the close of this cell generation, it is lost in the general cytoplasm of the daughter cells.

In the prophase of the second maiotic division (homotype) the same phenomena recur. When the homotype mitosis is over, the constituents of the sphere, or at least some of them, enter into direct relation with parts of the spermatozoon which arises by further differentiation of the cell. As regards the archoplasm, with which we are more directly concerned, it is again seen to contain a number of minute vesicles which continue as before to grow in size, whilst each contains a single refractive and stainable granule (figs. 4, 5). Subsequently, several of these vesicles fuse together, so that at a later stage in the metamorphosis of the cell into a spermatozoon there only remains a single large clear body, bounded by a distinct membrane, containing in the centre one or more darkly staining granules (figs. 6, 7, 8).

---

* Honda, 'Virchow's Archiv,' vol. 174.

† Borrel, 'An. Inst. Past.,' vol. 15. This author was on the right track in attributing importance to the archoplasm, but the erroneous interpretation placed on the centrosomes precluded his arriving at a satisfactory conclusion as to the nature of the bodies under discussion.

‡ Benda, 'Verh. deutsch. Gesellsch. f. Chir.,' 1902.

§ Moore, 'Internat. Monatschr. f. Anat. v. Physiologie,' vol. 11.

Fig. 4.

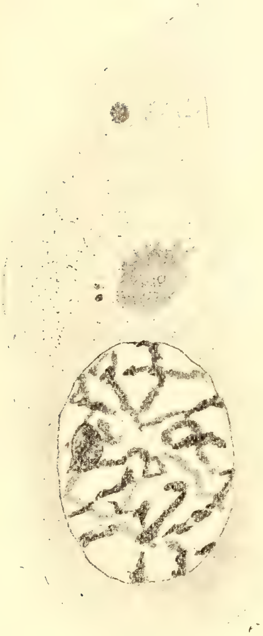


Fig. 5.

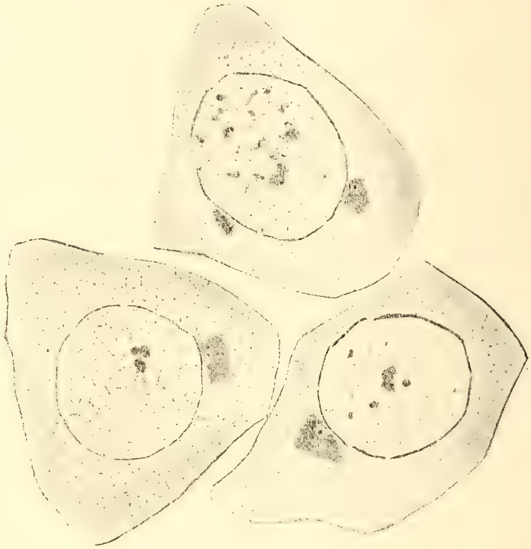


Fig. 4. Archoplasm with centrosomes lying outside it in prophase of the first maiotic division in testis of mouse.

Fig. 5. Spermatid of mouse, showing origin of vesicles in the archoplasm.

This body, originally described by one of us in 1895 as the archoplasmic vesicle, is a very conspicuous and apparently constant feature peculiar to the spermatogenetic cells of, at any rate, the vertebrata, and it has since been encountered beyond that group by other observers.

When fully developed it often assumes a size approximating to that of the nucleus. Indeed, the latter is often deformed and made to assume a crescentic or cuplike shape owing to the enlargement of the adjacent archoplasmic vesicle. The vesicle and its contents ultimately forms the so-called "cephalic cap" of the spermatozoon.

The remarkable similarity between the structure just described and those known as Plimmer's Bodies will have become obvious. It is not, perhaps,

Fig. 6.

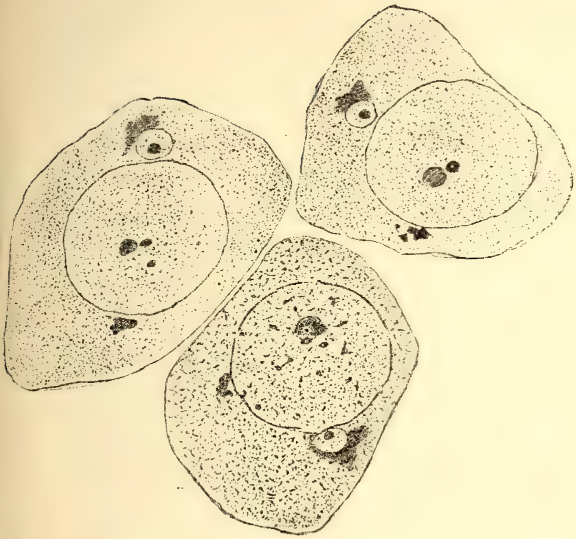
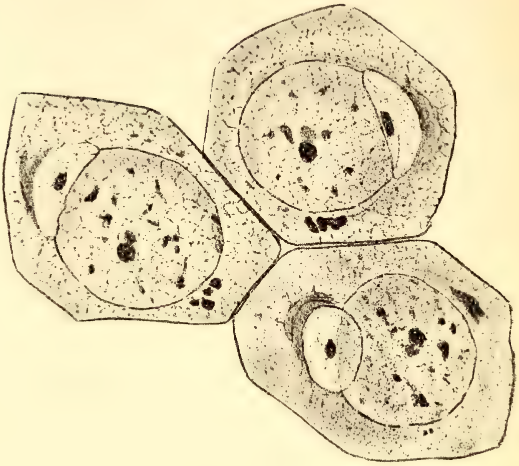


Fig. 7.



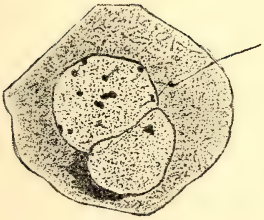


Fig. 8.

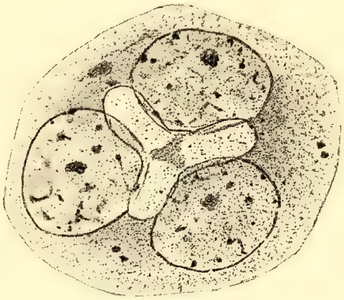


Fig. 9.

Figs. 6 and 7. Later stages of fig. 5.

Fig. 8. Slightly later stage in the spermatid of man, with centrosomes and tail.

Fig. 9. Three spermatid nuclei in a single cytoplasmic mass, showing three archoplasmic vesicles in the centre, with two pairs of centrosomes, and a third, less defined, to the left hand.

accidental that just as in the case of nuclear divisions, so also in the cellular inclusions, a parallelism between the cells of reproductive tissues and of cancer cells should be found to exist. But we do not on this account regard the cells of cancer as *identical* with those of the sexual cells, as we were careful to point out in our first communication in 1903.

But the resemblances between what we have termed gametoid, and the true gametogenic cells now seem to be even more significant than they appeared to be at that time. Both classes of cells are autonomous to a very high degree, and both possess the faculty of continuous or intermittent multiplication independently of the tissue requirements of the organism. And finally both exhibit cellular and nuclear metamorphoses which not only, *mutatis mutandis,* resemble one another, but differ materially from those pertaining to the normal somatic cells.

It is possible that the malignant elements are the outcome of a phylogenetic reversion, as was suggested by Sir William Collins, but the matter is obscured by the disturbing influences that have been operative during the actual ontogeny of the cells and tissues from which these elements have sprung. If this be so, the connection apparent between gametoid and the true reproductive cells will acquire a still deeper significance. But we propose to reserve the discussion of this question for another occasion.

In thanking those who have helped us with material we would mention especially Dr. Plimmer himself, who has most kindly placed preparations at our disposal.

We would further record our indebtedness to the Imperial Cancer Research Fund for a grant in aid of our investigations.

Observations on the Brains of Men and Animals infected with Various Forms of Trypanosomes. Preliminary Note.

By F. W. Mott, M.D., F.R.S.

(Received March 14,—Read March 16, 1905.)

The material upon which these observations are based has been forwarded to me from Uganda, with the exception of the brain of a rabbit kindly given to me by Dr. Plimmer.

By the desire of the Committee on Tropical Diseases of this Society, Colonel Bruce has given instructions to his assistants, and they have forwarded to me from Entebbe, material from cases of Sleeping Sickness, 24 in all; also portions of the brains of eight monkeys experimentally infected, two oxen, and one donkey.

The tissues have been preserved in Formol-Müller's solution, or they have been sent already embedded in paraffin after having been hardened a short time in formalin. Sections were cut of either 5 μ or 10 μ in thickness; they were stained by Romanowsky, Leishman, Nissl polychrome, or Weigert-Gram methods, thus enabling observations to be made regarding:—

(*a*) The existence of trypanosomes or Leishman bodies.

(*b*) Changes in the ganglion cells and neuroglia cells.

(*c*) Changes in the blood, the endothelial cells of the vessels, and the peri-vascular spaces, and soft membranes.

(*d*) The existence of micro-organisms.

(*e*) The existence of plasma cells, and other cells indicative of chronic inflammatory degenerative changes.

It may be mentioned that in a number of instances sections of the lymphatic glands, some of which were removed during life and others *post-mortem*, were examined by the same methods. The principal pathological conditions observed were either drawn or photographed.

In every case of Sleeping Sickness, in which there were symptoms during life, the characteristic change (which I described in two cases reported by Sir Patrick Manson) of a chronic meningo-encephalitis was found. In every chronic case I have found also plasma cells of Marscholko, which by some observers were considered to be pathognomonic of another chronic meningo-encephalitis, general paralysis of the insane.

Every stage of the development of these plasma cells from lymphocytes can be observed in the brain tissues, also in the chronically inflamed lymphatic glands. Likewise, in chronic cases, morular cells, indicative of chronic inflammation, can be seen in most cases.

From the clinical notes furnished in these cases, it is apparent that there is a correlation between the severity of the symptoms, the chronicity of the disease and the degree and intensity of the chronic inflammatory process, as evidenced by the abundance of lymphocytes in the peri-vascular spaces and in the subarachnoid space, also by the number of plasma cells and morular cells.

With regard to the ganglion cells, the very chronic cases show a very marked degeneration of the ganglion cells of the central nervous system, particularly of the medulla oblongata and the cortex—proportional to the degree of affection of the vessels—many of the smaller of which are completely occluded, partly by the accumulation of lymphocytes, partly by the proliferation of the nuclei of the endothelial cells. Many of the capillaries are completely occluded by this process, and the result is not merely a chromatolysis of the ganglion cells, but a coagulation necrosis and destruction. Where there is this advanced degeneration, there is a marked proliferation of the glia cells, and a large number of spider cells can be seen; in fact, the appearances, as the accompanying pictures show, closely resemble, in many respects, the chronic inflammatory changes met with in general paralysis. Except that, whereas in the latter disease, the vascular change is in great measure secondary to the degenerative change of the ganglion cells; in Sleeping Sickness the chronic inflammatory process is universal throughout the central nervous system, and the ganglion cells are destroyed, secondarily to the occlusion of vessels.

The glia cell proliferation is not nearly so pronounced in Sleeping Sickness, because there is so much less wasting of the brain substance. In one case, so marked was the peri-vascular infiltration in the grey matter of the cortex, that after hardening in Müller's solution the vessels could be seen with the naked eye as glistening pearly lines and points.

In the blood contained in the vessels of a few of the chronic cases without micro-organismal infection, a solitary trypanosome or a portion of a trypanosome could very occasionally be seen, but it is a remarkable fact how very seldom, in the immense number of sections examined, I have been able to find evidence of trypanosome infection by examination of the blood contained in the vessels; I have, therefore, concluded that these organisms cannot be abundant, and that if they produce this chronic inflammation as all the facts in the etiology of the disease prove, it may either be that they

(1) produce a toxin which acts as the irritant; (2) they *undergo morphological changes* in the blood or cerebro-spinal fluid; or (3) that the secondary or terminal infection with which nearly all these cases were affected (except three), with diplococci, diplo-streptococci, or occasionally other organisms such as cocci, Friedlander's bacillus and bacillus coli, leads to the destruction of the trypanosomes.

It is probable that the defences of the organism against bacterial invasion are lowered by the trypanosome blood infection. It has been shown that recently a large number of natives have been dying of pneumonia. The diplococcus is one of the most prevalent organisms found in the body. Again, negroes, owing to jiggers and other sources of infection by pyogenic organisms have therefore ready to hand a source of secondary or terminal infection.

A very interesting case in this respect was Bara Risgalli; this man for a long time had infection with *Trypanosoma Gambiense* in the blood; these organisms were also obtained from his lymphatic glands by Captain Greig. Sections of the glands removed during life were examined by me, and I found evidence of degenerated trypanosomes, macro-nuclei and micro-nuclei; also the glands showed marked evidence of chronic inflammatory change; plasma cells and degenerated cells being abundant. The gland removed during life was sterile, that is free from micro-organisms, and no diplococci could be discovered in the sections which I examined. At this time, he showed no signs of Sleeping Sickness; later he was taken ill, and as I learnt from Captain Greig, he died in 10 days of pneumonia with cerebral symptoms.

Examination of the brain showed a well marked acute pneumococcic meningitis; in fact, I should not have thought of Sleeping Sickness upon looking at the sections without prejudice, for the leucocytic infiltration was almost entirely confined to the membranes, consisted almost entirely of polymorphonuclears, and it did not extend into the peri-vascular spaces. Amid and within the leucocytes were immense numbers of diplococci with capsules; the lymphatic glands, which were previously sterile, now all contained diplococci. Whether this man would in time have developed the chronic meningo-encephalitis of Sleeping Sickness and its associated progressive phenomena, I am unable to say; but it is an interesting point in connection with the fact that some authorities look upon the *Trypanosoma Gambiense* as a distinct form from that which produces Sleeping Sickness.

The European case, reported by Sir Patrick Manson, of a missionary's wife who died in England with the lesion of Sleeping Sickness, having suffered for some time with trypanosome fever, and with *Trypanosoma Gambiense* in the blood, is opposed to the view of a distinct organism. It is possible, therefore, that Bara Risgalli, had he lived longer, would have developed Sleeping Sickness, for in some places a few of the vessels showed slight lymphocyte proliferation in the surrounding lymph spaces; but even this is not conclusive evidence, for I have found the same in chronic diplo-streptococcic meningitis.

There is another man, Tabula, whose glands, removed *intra vitam,* I have examined, and which showed exactly the same changes as Bara Risgalli, and who, I understand, has similar trypanosomes in the blood, and general glandular enlargement, but has not yet developed any signs of Sleeping Sickness. It will be interesting to see what becomes of him.

It has been shown that the cerebro-spinal fluid in Sleeping Sickness always contains trypanosomes, and likewise the juice of the lymphatic glands by puncture during life. On examination of *sections* of the glands in a number of these cases, in which active trypanosomes had been found during life, I observed only rarely a body which I could definitely call a trypanosome, therefore, it is not surprising that I was unable to find, after very careful search of many hundreds of sections, any body which I could definitely recognise as a trypanosome in the meningeal peri-vascular cell infiltration of the central nervous system. Yet, as the coloured drawings show, not only did one see similar cells and products of chronic inflammatory change in the peri-vascular lymph spaces, but also similar products of degeneration and similar staining chromatin bodies and bits like those seen in the lymphatic glands, which we have reason to believe may be products of degenerated trypanosomes. Moreover, I have occasionally seen a macro-nucleus with its accompanying micro-nucleus amidst the cells of the peri-vascular infiltration. In the lymphocytes themselves, in a chronic case in which I could discover no diplococcal infection by Gram's method, I have found deep staining bodies, oval or round in shape, but I am unable to affirm what they are.

If the trypanosomes are continually being destroyed, as they seem to be by the cells, it is not surprising that more evidence of their existence is not seen. It is remarkable in transections of the blood vessels in very chronic cases, to observe how few are the polymorpho-nuclear leucocytes, and how numerous the small and large mono-nuclears, and apparently these get into the peri-vascular spaces.

Examination of tissues of other organs from cases of Sir Patrick Manson's showed that not only the brain and glands are affected, but serous membranes and organs of the body, by this lymphocyte infiltration around the vessels, although to a much less degree.

Examination of the Brains of Animals Infected with Trypanosomes.

It has been stated that trypanosomes cannot be shown in sections of the brain, and that the hardening fluid may have been the reason why more definite evidence of the trypanosome infection in sleeping sickness has not been observed. The following observations show that both these hypotheses are probably untenable.

(1) The brain of a rabbit inoculated with *Surra*, which died three months later, hardened in formol, was kindly given me by Dr. Plimmer, and showed the following appearances in sections. By any of the staining methods employed, nearly all the blood vessels showed masses of trypanosomes, as the coloured drawings exhibit. Single trypanosomes could be seen in the capillaries; in the larger vessels solitary trypanosomes, and whorls of trypanosomes, and plasmoidal masses, which may either be degenerated trypanosomes consisting of a zooglœal mass, in which more deeply stained macro-nuclei and micro-nuclei can be seen, or, as Plimmer and Bradford consider, of amœboid forms. But in spite of this extraordinary trypanosome infection, the blood vessels showed little or no inflammatory reaction. The peri-vascular spaces showed no lymphocytes, the ganglion cells showed marked chromolytic changes, otherwise there was nothing noteworthy in the nervous system.

(2) The brains of two oxen infected with Jinga trypanosomes were examined. The animals died within three months of infection; the results of the examinations were extremely interesting and will be given in some detail.

Experiment 162.—The cortex cerebri, the cerebellum, medulla and spinal cord were examined, and all yielded the same results. With a magnification of 1200 diameters, the capillaries and vessels were found to contain chromatin bodies, exactly resembling Leishman bodies, except that they were smaller, measuring from 1 to 2 μ, much more frequently 1 μ, rarely as large as 2 μ. They were either circular or oval rings or had the appearance of the chromatin particles being situated at the two poles. Several drawings and photographs are given to illustrate their appearance and their numbers. Some of the capillaries show immense numbers, and in some transections of larger vessels, these bodies can be observed lying in a zooglœal mass.

Individual bodies exhibit some diversity in their form, indicating division. A large number of stained particles (which may be micro-nuclei) can be seen.

The Jinga trypanosome, as the accompanying drawing shows, is comparatively a large organism, as seen in the blood of a monkey, which was inoculated with it. Its oval macro-nucleus is much larger than these chromatin bodies which are seen in the vessels. If these chromatin bodies, as Leishman would affirm, are the macro-nuclei of trypanosomes, then it is difficult to explain why a dozen or more of the chromatin bodies can sometimes be seen lying in a space which would be covered by one trypanosome. Still the trypanosomes may have degenerated elsewhere, and the macro-nuclei be carried into the capillaries. In view, however, of the researches of Captain Rogers regarding Leishman bodies being altered phases of trypanosomes, and the contention of Plimmer and Bradford *re* the existence of amœboid forms of trypanosomes, it is possible that these chromatin bodies may be some phases in the life of the trypanosome in the blood; and it may be mentioned in support of this (although I do not profess to dispute the opinion of biologists who have studied the question) that after a very careful search of a large number of sections, I have been unable to see a single trypanosome or a degenerated one, which is quite different to what one found in the brain of the rabbit inoculated with *Surra.*

Experiment 202. *Ox.*—This animal died within three months of infection, the same portions of brain were examined. It was only after some careful searching that I could find a few small vessels containing these chromatin bodies. A drawing is given to illustrate these vessels. It will be observed that there are a far larger number of minute, just visible, stained particles.

Addendum.—Since reading this preliminary note, by a new method of staining I have found trypanosomes, and what I believe are Plimmer and Bradford's amœboid forms in ox, Experiment 202.

Clearly, then, at any rate in the brain, the evidence of the existence of trypanosomes in the blood of animals dying of trypanosome disease may vary very considerably. In view of the fact that blood in which no trypanosomes can be detected microscopically, yet by culture experiments they may be obtained, it may be asked (although here, again, I do not pose as an authority) whether these bodies with chromatin particles can develop into trypanosomes.

In the vessels of the brain of the Ox 162, many leucocytes can be seen which have taken up the chromatin bodies. It may be mentioned that in these two cases there is no sign of meningo-encephalitis, and there was no diplo-streptococcal infection. The ganglion cells showed chromolytic changes, and there were *many minute capillary* hæmorrhages, probably due to plugging of the capillaries by the organisms.

Moreover, there were curious cells lying free in the vessels, which, however, I could not assert were not detached endothelial cells with nuclear changes, except that I have not observed such appearances before. (*Vide* drawing.)

Donkey inoculated with mule trypanosomes. In this case the central nervous system yielded no positive results.

Monkeys inoculated with different varieties of trypanosomes (including four certain cases of Sleeping Sickness), of which eight brains have been examined. The animals lived for varying periods, from a month or two to over one year. As the results were negative, I shall not give any particulars here.

The most obvious change found was the empty condition of the small blood vessels and chromolytic changes in the nerve cells. There was no perivascular cell-infiltration and no meningitis. The tissues of some of them showed diplococci.

In one case of Sleeping Sickness (Zurura Mya), a chronic case in which trypanosomes were found in the cerebro-spinal fluid during life without centrifuging, I was unable to find any perfect trypanosomes in sections of the central nervous system, but I found numbers of bodies which I thought might be altered forms, or fragments of degenerated trypanosomes and chromatin bodies, especially in the chronic inflammatory exudation of the subarachnoid space. Moreover, small capillaries could be found ruptured in the neighbourhood. This fact I have observed in other chronic cases, and suggests the possible mode of infection by trypanosomes of the cerebro-spinal fluid in the subarachnoid space.

I have had the opportunity, recently, of examining sections of a case of chronic basal meningitis with diplo-streptococcal infection. Sections showed in places a very marked peri-vascular infiltration of some of the vessels of the cortex, away from the primary source of infection, resembling, in some respects, some of the less chronic cases of Sleeping Sickness. I failed, however, to discover, amidst the cell exudution, those small round and oval bodies and fragments which I have found in the meningo-encephalitis of chronic Sleeping Sickness. But, in a case of basal meningitis occurring in a child, only the membranes about the base of the brain were affected, and no perivascular infiltration was found. There were numbers of small round and oval bodies, probably products of degenerated cells.

In my opinion, therefore, a series of culture experiments *in vitro* of different forms of trypanosomes, especially of "Sleeping Sickness" in cerebro-spinal fluid, would be of interest. This fluid, containing a mere trace of proteid, might lead to degeneration of these organisms, and products and fragments, similar to those found in the membranes, might be observed on microscopical examination of the centrifuged fluid. If no change in the trypanosomes occurred, infection of the cerebro-spinal fluid by diplo-cocci or diplo-streptococci might be undertaken.

Positive results by this method might help in deciding this difficult point; whether the chronic inflammatory exudation is the result of the irritation caused by the trypanosomes or of their toxic products; and whether any of these small round and oval bodies, seen in great abundance in the chronic inflammatory products, are products of degenerated trypanosomes.

Addendum.—A full report of this investigation, with the abstract of the clinical notes of the cases, the photo-micrographs and drawings, will appear in the Reports of the Sleeping Sickness Commission.

By the kind permission of Major Leishman, I have since had the opportunity of examining a portion of the cortex cerebri of a monkey which died quite recently; this animal was inoculated with the blood of a case of Sleeping Sickness 18 months previously, and unlike any of the eight monkeys' brains which I have had the opportunity of examining, it shows a well-marked meningo-encephalitis. This very important fact was referred to by Major Leishman in the discussion that ensued, and a full report of the examination of the brain will be published by Captain Harvey.

REFERENCES.

"The Examination of the Tissues of the Case of Sleeping Sickness in a European," by Geo. C. Low, M.A., M.B., and F. W. Mott, M.D., F.R.S., 'Brit. Med. Journal,' April 30, 1904.

"The Leishman-Donovan Body and Tropical Splenomegaly," by Sir Patrick Manson and Dr. Low, 'B. M. J.,' January 23, 1904.

"Preliminary Note on the Development of Trypanosoma in Cultures of the Cunningham, Leishman-Donovan Bodies of Cachexial Fever and Kala-azar," by Leonard Rogers, M.D., M.R.C.P. Lond., Capt. I.M.S., 'Lancet,' July 23, 1904.

"The *Trypanosoma Brucei*, the Organism found in Nagana, or Tsetse Fly Disease," by J. R. Bradford, F.R.S., and H. G. Plimmer, F.L.S., 'Quart. Journ. Micr. Sci.,' vol. 45, April 20, 1905.

On a New Rhabdosphere.

By George Murray, F.R.S.

(Received January 13,—Read February 23, 1905.)

After the publication by Mr. Blackman and myself in the 'Phil. Trans.'* there appeared in the 'Zoologischer Anzeiger'† a tract by Professor Ostenfeld, of Copenhagen, on the Coccospheres and Rhabdospheres. His main idea was to split up the Rhabdospheres (so far as his treatise concerned the Rhabdospheres) into two genera, viz., *Rhabdosphæra* of Sir John Murray and *Discosphæra* of Haeckel.

From the beginning I distrusted Professor Ostenfeld's discrimination as I had distrusted Professor Haeckel's, and it has now been my fortune to discover a new Rhabdosphere which destroys, in my opinion, the idea of breaking up the genus *Rhabdosphæra.*

Of the species of this genus, the most elusive organisms in natural history, there have been but few good specimens. They were derived in the first place, in broken-down fragments, from deep-sea deposits, and conjectures were made as to their origin. The next stage was the description of the Rhabdospheres as surface organisms by Sir John Murray during the "Challenger" Expedition, and still later, more completely as I hope, in the 'Phil. Trans.,'‡ by Mr. Blackman and myself. There are only two forms known, and I now propose to add a third. The interest is not only systematic, but may have bearings§ on geological points and also on the study of deep-sea deposits, which is very much the same problem.

I desire to associate the name of my colleague, Mr. V. H. Blackman (my companion in many arduous sea journeys) with this Rhabdosphere, and I propose, therefore, its name shall be *Rhabdosphæra Blackmaniana,* G. Murr.

Its outstanding characteristic is the possession of sharp spinous processes, in contrast to the trumpet-shaped and club-shaped processes of the two known species.

The points of general interest are these, viz., the novelty and extreme rarity of the Rhabdosphere, and the fact of my never having once met with it in the deep-sea deposits or geological specimens so kindly put at the disposal of Mr. Blackman and myself by Professor Judd. The minuteness and extreme tenuity of the spines probably amply account for its not having been recognised in the deep-sea deposits.

---

* B, vol. 190, 1898.

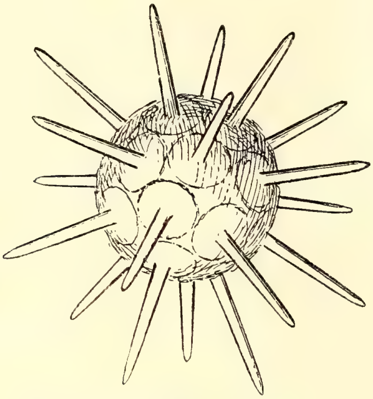
† Vol. 23, No. 612, April 9, 1900.

‡ *Loc. cit.*

§ *Cf.* 'Phil. Trans.,' B, vol. 190, 1898.

The formal description is as follows :—

Rhabdosphæra Blackmaniana, n. sp. Very minute, 10 μ, *i.e.*, about one quarter the size of *R. claviger*, with tapering acute short spinous processes.



Rhabdosphæra Blackmaniana, n. sp.

Lat. obs. 28° 25′ S., long. 23° 56′ W. On the outward voyage to the Cape of the "Discovery."

I add no greater details, since the subject of Rhabdospheres has been dealt with so fully by Mr. Blackman and myself.*

---

* *Loc. cit.*

On some New Species of Lagenostoma, a Type of Pteridospermous Seed from the Coal Measures.

By E. A. Newell Arber, M.A., F.L.S., F.G.S., Trinity College, Cambridge, University Demonstrator in Palæobotany.

(Communicated by D. H. Scott, F.R.S. Received January 10,—Read February 23, 1905.)

[Plates 1 and 2.]

CONTENTS.

PAGE
Introduction 245
A. *Lagenostoma Kidstoni*, sp. nov.—
(1) History, Locality, and Horizon of the specimens 247
(2) Morphology of the seed 247
(3) Position of the seed on the plant 250
(4) Summary.. 251
B. *Lagenostoma Sinclairi*, Kidston M.S.—
(1) History, Locality, and Horizon of the specimens 251
(2) Morphology of the seed and "cupule" 252
(3) Position of the seed on the plant 253
(4) Summary.. 254
General conclusions.. 255
Explanation of the plates 258

Introduction.

The recent discoveries of the female fructifications of more than one member of the Cycadofilices mark an epoch in the history of the study of this great Palæozoic group, and give a valuable clue to the phylogeny of the Cycads, an at one time numerous and important class of Spermophytes, some of which still survive at the present day.

It is somewhat remarkable that the discoveries of the seeds of two such diverse genera as *Lyginodendron* and *Medullosa* should have followed so closely on one another. In May, 1903, Professor Oliver and Dr. Scott* showed that a petrified seed of the Gymnospermous type, previously known as *Lagenostoma Lomaxi*, Will. M.S., belonged to *Lyginodendron*, a genus of Cycadofilices possessing much divided fern-like foliage of the *Sphenopteris* type. In the December of the same year, Mr. Kidston* described casts of a radiospermic seed in organic continuity with pinnules of *Neuropteris heterophylla,* a type of foliage which there is every reason to believe belonged to certain Palæozoic stems known as *Medullosa.* This seed he identified as closely similar to that first described by Gœppert and Berger as *Rhabdocarpus.*

---

* Oliver and Scott, "On *Lagenostoma Lomaxi*, the Seed of *Lyginodendron*," 'Roy. Soc. Proc.,' vol. 71, p. 477, 1903; and "On the Structure of the Palæozoic Seed *Lagenostoma Lomaxi*, with a Statement of the Evidence upon which it is referred to *Lyginodendron*," 'Phil. Trans.,' B, vol. 197, p. 193, 1904.

These discoveries have removed any possible doubt which remained as to the correctness of the conclusion that the stems, which bore such fern-like foliage as *Sphenopteris* and *Neuropteris,* exhibit in their anatomy characters foreign to the true ferns, but common to the Gymnosperms.

As a corollary of this recent work, attention has been called afresh to the seed-like bodies which occur here and there, as casts or impressions, in the shales and sandstones of the Coal Measures. It would seem probable that since impressions of Sphenopterid fronds, some of which there is reason to believe represent the foliage of members of the Cycadofilices, occur fairly abundantly in these rocks, impressions or casts of seeds similar to *Lagenostoma* should also be occasionally found. The more so as several species of the much larger type of seed, shown by Mr. Kidston to belong to a plant of the Neuropteroid habit, are already known to science. So far, *Lagenostoma* has been recorded only in the petrified state, *i.e.,* in a condition in which the anatomical structure is preserved. But further search has been rewarded by the discovery of what are believed to be two new species, occurring as casts or impressions, which are described here. These two species indirectly throw light on the habit of *Lyginodendron,* especially with regard to the manner in which the female organs were borne; a character which is, with rare exceptions, extremely difficult to ascertain in the case of fossil plant-remains, owing to the fragmentary nature of the evidence.

I may here express my sincere thanks to Dr. Scott, F.R.S., who has most kindly given me the benefit of his advice at every stage in the progress of this work, and to whom I am greatly indebted for valuable suggestions in regard to the interpretation of these specimens.† I am also indebted to him for his kindness in communicating this paper. My thanks are also due to Mr. L. A. Boodle, for three of the photographs reproduced on Plate 1.

---

* Kidston, "On the Fructification of *Neuropteris heterophylla,* Brong.," 'Roy. Soc. Proc.,' vol. 72, p. 487, 1903; 'Phil. Trans.,' B, vol. 197, p. 1, 1903.

† A preliminary note on these specimens by Dr. Scott, in conjunction with the present writer, will be found in the forthcoming volume of the Report of the Cambridge meeting of the British Association. Owing to the number of papers proposed at that meeting, this note was not communicated at the time.

A. *Lagenostoma Kidstoni*, sp. nova.

(1) *History, Locality, and Horizon of the Specimens.*

Among the collection of Carboniferous plants in the British Museum (Nat. Hist.), there is a large triangular slab of shale,* bearing numerous casts and impressions of seeds, for which the name, *Lagenostoma Kidstoni*, is proposed. The specimen in question is merely recorded as from the Glasgow Coalfield. I am, however, indebted to Mr. Kidston for information as to the locality and horizon from which it was obtained. Mr. Kidston was aware of the existence of a precisely similar specimen in the Hunterian Museum, Glasgow, which he kindly identified, and through the good offices of Professor Graham Kerr and Dr. Scott, I obtained the loan of it for purposes of examination. The Glasgow specimen, which was presented to that museum in 1867 by Mr. John Smith, bears a full record of the horizon and locality. It was collected from the Lower Coal Measures, at Swinhill Colliery, Stonehouse, Lanark, from the horizon of the Virtue Well Coal. As has been stated, it is precisely identical with the specimen in the British Museum, and there is no reason to doubt that both specimens were obtained from the same bed, especially as Mr. Kidston possesses information confirming this conclusion.

I have much pleasure in naming this species in honour of my friend, Mr. Kidston, to whom I am greatly indebted for the trouble he has taken in this matter. I may also here express my thanks to Professor Graham Kerr, and to Dr. Smith Woodward, Keeper of the Geological Department of the British Museum, for facilities for studying these specimens.

I regard the specimens at London and Glasgow as co-types.

(2) *Morphology of the Seed.*

On both the slabs of shale preserved in the British Museum, and at Glasgow, casts or impressions of *Lagenostoma Kidstoni* are extremely numerous, and they are the only seeds to be observed on these specimens. Slightly enlarged photographs of the Glasgow example are reproduced on Plate 1, photos. 1, 3, and 4; the remaining photograph on the same plate (photo. 2) being taken from the British Museum slab. In the great majority of cases the seeds are preserved as casts.

The seeds measure on an average 6 mm. in length, and, at their greatest width, between 2·5 to 3 mm. in breadth. They are nearly all of the same size. As compared with the three known species of *Lagenostoma*, *L. Kidstoni* is about the same length as fully-mature specimens of *L. Lomaxi*† and *L. physoides,*[*] but considerably longer than *L. ovoides.*[†] *L. Lomaxi,* is, however, a much broader type of seed. On the whole, the new species stands nearest to *L. physoides* in point of size.

---

* Registered number in the Geological Dept., V 6241.

† Oliver and Scott, *ibid.*, p. 198.

The seeds are undoubtedly of a radially symmetrical type, or radiospermic. They are elliptical in shape (Plate 2, figs. 1 to 3), and broadest midway between the apex and the chalaza. The apex is a little more pointed than the base. There is no reason to doubt that they were borne as sessile bodies on an axis. The hilum is seen in a specimen figured on Plate 2, fig. 2.

The integument is smooth, and in instances where the carbonaceous film covering the seeds is preserved, it is characterised by numerous, longitudinally arranged, minute dots, having thus a punctate appearance (*cf.* enlarged drawings on Plate 2, figs. 1, 2, and 3). Many of the seeds, especially those which appear to be less compressed, show several slight longitudinal ridges or keels. The number of keels appears to equal the number of apical lobes, and they traverse the seed in such a manner as to end at the apex of each lobe. These keels are conspicuous in photos. 1, 3, and 4 on Plate 1, and are also seen in the enlarged drawing on Plate 2, fig. 3. Somewhat similar ridges have been described in the seed, *Lagenostoma Lomaxi.*[‡] In instances in which the preservation is particularly good, the rectangular form of the cell walls of the integument can be seen under a high magnification. The appearance of these cells is shown on Plate 2, fig. 6, where they are magnified about 70 diameters.

The lobed nature of the integument at the apex of the seed at once distinguishes *L. Kidstoni* from any other seeds, preserved as casts or impressions, which have been previously described. In view of Williamson's[§] description of the structure of *Lagenostoma physoides,* there can be no hesitation in identifying this seed as a member of that genus, although the structure in this instance is not preserved. In this conclusion I am confirmed by the opinion of Professor Oliver, who has made a special study of the Palæozoic petrified seeds, and who has very kindly examined this material.

The enlarged drawings of selected seeds reproduced on Plate 2, figs. 1 and 3, show that the apex is divided into several short, blunt lobes. The apical lobes are also clearly seen in several of the photographs on Plate 1, more especially in photos. 3 and 4, and less distinctly in photo. 1. It is difficult to judge of the exact number of lobes in such a cast, in which only one side of the seed is visible, but in several instances (Plate 2, fig. 3) in which the apex of the seed is particularly clear, there appear to be six lobes. The number is not, however, necessarily constant. In *L. physoides*, the species which stands nearest to that under discussion, the number varied from 10 to 11, and in the other species, *L. Lomaxi* and *L. ovoides*, there are indications that the number of the fluted ridges at the apex, corresponding to the lobes in *L. physoides* and *L. Kidstoni*, was not always identical in every specimen. It is known from the investigations of petrified material of similar seeds that these lobes or flutings correspond to the chambers of a dome-like structure, the canopy,* which surrounds the pollen chamber.

---

[*] Williamson, "On the Organisation, Part VIII," 'Phil. Trans.,' vol. 167, p. 241, Plate 61, figs. 77—78, Plate 62, fig. 79, 1877; and Butterworth, "Some further Investigation of Fossil Seeds of the genus *Lagenostoma,* etc.," 'Mem. and Proc. Manchester Lit. and Phil. Soc.,' vol. 41, Part 3, Mem. IX, Plate 8, 1897.

[†] Williamson, *ibid.*, p. 234, Plate 59, figs. 53—59, Plate 60, figs. 60—70 and 74—75 Plate 61, figs. 70, 72—73.

[‡] Oliver and Scott, *ibid.*, p. 198.

[§] Williamson, *ibid.*, p. 241, Plate 2, fig. 77; also Butterworth, *ibid.*

Of the hundreds of these seeds which have been examined, whether in the Glasgow or the British Museum specimens, every one has proved to be naked. At the stage of their development at which they are there represented—a stage which appears to be the same in practically every instance—there is reason to believe that the seed was not enclosed in any protective organ, similar to that described as the "cupule"† of *L. Lomaxi*, which played such an important *rôle* in the correlation of that seed with *Lyginodendron*. In only one instance (Plate 2, fig. 4; Plate 1, photo. 4) has the presence of any structure been detected which could be regarded as of this nature, and here the organ in question does not obviously subtend, although it is in close proximity to a seed. The preservation is not particularly good and its form is not very clear. It appears to consist of a circular or oval ribbed-sheath, probably of a foliar nature, divided distally into a large number (considerably more than 15) of rather blunt, lanceolate teeth, each about 0·5 mm. in length. The length of the whole sheath is about 5 mm.

Although there is no Upper Carboniferous foliar organ with which I am acquainted, that at all agrees with this sheath-like body, I am not disposed to attach any special importance to its occurrence here in the neighbourhood of a detached seed. It may be that the seeds of *L. Kidstoni*, like those of *L. Lomaxi* and *L. Sinclairi*, the latter to be described here subsequently, were enclosed in a "cupule" at an earlier stage in their development than that represented in these specimens, and that, like *L. Lomaxi*, the mature seeds may in most cases have been naked. On this assumption, the organ under discussion might be interpreted as of the nature of a "cupule." This single instance, however, hardly furnishes reliable evidence on this point, and for the present we can only conclude that these seeds were generally naked at the stage of their development represented in these specimens.

---

* Williamson, *ibid.*, p. 235; Oliver and Scott, *ibid.*, p. 203.

† Oliver and Scott, *ibid.*, p. 215.

(3) *Position of the Seed on the Plant.*

In the photographs of the two specimens reproduced on Plate 1, it will be noticed that the seeds are in most cases obviously detached. Associated with them are several long, naked, rachis-like structures, seen in Plate 1, photos. 1 and 3, etc. Some of these axes are 25 cm. or more in length, and vary from 3 to 13 mm. in breadth. The surface as a rule is striated longitudinally, the striæ being fine and regular. In addition, very minute, crowded circular or oval pits, or in other cases slight prominences occur, and these are arranged more or less in pseudoparallel longitudinal rows along the median portion of the axis (Plate 1, photos. 1 and 3). In some instances, these rachis-like structures have been observed to branch.

In no case has any foliar organ been found attached to these branched axes. In their general characters, however, they may be somewhat closely compared with portions of highly-compound fronds of the *Sphenopteris* type. There are strong reasons, as will be shown at a later stage in this work (p. 254), for regarding these branched structures as of the nature of compound fronds with reduced lamina, belonging to the same genus *Sphenopteris*. This conclusion is founded mainly on the evidence of the seed itself, and that presented by *L. Sinclairi,* of which a description is included here, in addition to the characters of these rachis-like axes.

At first sight there appear to be several instances, in both the British Museum and Glasgow specimens, in which the seeds are still attached to these axes. This is apparently the case in Plate 1, photo. 4, a portion of the Glasgow specimen. A careful examination of this and similar examples has, however, shown that in no case is there any real evidence of continuity, and I regard these instances as probably due to chance association of detached seeds with the axis-like structures. In dealing with such impressions it is necessary to be exceedingly guarded on the subject of continuity, for such association is often capable of a totally different explanation. In only one case does it seem probable that these seeds may be still attached to the axis on which they were borne in the living state, and here the axis appears to be the termination of one of the finer branches of these rachis-like structures. This specimen is figured on Plate 2, fig. 5, enlarged three times. Here two seeds terminate the axis, which may very possibly be still in continuity, although even this instance may not be entirely free from doubt.

If this specimen is rightly interpreted, there would appear to be some evidence, though not as conclusive as one could wish, for the provisional view that the seeds, described here as *Lagenostoma Kidstoni*, were borne sessile on the terminations of the finer branches of a foliar organ of the *Sphenopteris* type, which is probably best interpreted as a frond with reduced lamina.*

Summary.

The chief conclusions with regard to the new seed, *Lagenostoma Kidstoni*, may be summed up as follows:—

The seed is elliptical in shape, averaging 6 mm. in length, and 2·5 to 3 mm. at its greatest breadth. It is undoubtedly radiospermic. The surface of the testa was smooth, and the seed was slightly ridged longitudinally.

The integument at the apex is divided into several lobes, probably six in number in most cases. In the condition of the integument at the apex, the seed agrees closely with *Lagenostoma physoides*, Will. The number of keels, or longitudinal ridges of the testa, probably equalled the number of apical lobes.

The seeds are naked in these specimens, and in all probability at this stage of their development were not enclosed in a "cupular" investment.

It is provisionally suggested that they were borne in a sessile manner on the finer terminations of a foliar organ, probably of the nature of a compound frond of a *Sphenopteris* with reduced lamina.

B. *Lagenostoma Sinclairi*, Kidston M.S.

(1) *History, Locality, and Horizon of the Specimens.*

For the loan of the specimens of this *Lagenostoma*, I am indebted to Mr. Kidston, who recorded,† and intended to have described them, but he has very generously placed them at my disposal for description in relation to the other new species already discussed. I may here express my thanks to Mr. Kidston for his kindness in this matter.

The specimens‡ are of Lower Coal Measure age, and were obtained from Grange Colliery, Kilmarnock, Ayrshire, on the horizon of the Stranger

---

* This conclusion was briefly referred to by Dr. Scott in a lecture delivered in May, 1903. *Vide* Scott, "The Origin of Seed-bearing Plants," 'Royal Inst.,' May 15, 1903, p. 13.

† Kidston, "Some Fossil Plants collected from the Ayrshire Coalfield by Mr. A. Sinclair," 'Annals, Kilmarnock Glenfield Ramblers' Society,' No. 4, 1901—1904, p. 14.

‡ Registered numbers 3529—3531 in Mr. Kidston's collection. I regard these specimens as co-types.

Coal. They were collected by Mr. A. Sinclair, of Kilmarnock, in 1889, who also obtained a further specimen in 1903.

Mr. Kidston recorded these specimens as *Lagenostoma* sp., but he has since proposed in M.S., in honour of the collector, the specific name, *L. Sinclairi*, which I have pleasure in adopting here.

(2) *Morphology of the Seed and "Cupule."*

As will be seen from the drawings on Plate 2, figs. 7 to 11, the special interest of these specimens lies in the fact that many of the seeds are still enclosed in their protective envelopes, which are regarded as similar, morphologically, to the organ recently described as the "cupule" of *L. Lomaxi*.* The presence of a "cupule" naturally renders an exact description of the seed itself a somewhat difficult matter, but in some instances the "cupule" has apparently perished, and the seed is thus disclosed. We may first consider what can be made out with regard to the morphology of the seed.

The seeds appear to be somewhat smaller than in the case of *L. Kidstoni*. They vary from 4 to 5·5 mm. in length, and at their widest part from 1·5 to 3 mm. in breadth. In shape they are elliptical-oblong. They are undoubtedly radially symmetrical (Plate 2, fig. 9). The integument is apparently smooth, and slightly ridged longitudinally. There do not appear to be any lobes at the apex as in *L. Kidstoni*, but the apex is slightly notched or fluted (Plate 2, fig. 9), recalling in this respect *L. Lomaxi*. It is impossible in these specimens to determine the number of the longitudinal ridges, or of the flutings at the apex, but the apical characters, in conjunction with the general morphology of the seed, and the presence of a "cupule," leave little doubt that they may be correctly assigned to the genus *Lagenostoma*.

The "cupule," which encloses many of the seeds, varies from 8 to 9·5 mm. in length. The average breadth of the seed including the "cupule" is about 3 mm. at its widest part. The "cupules" appear to be attached to the axis slightly below the seed. They are sac-like organs, prominently ridged longitudinally, and entire for a distance of 5 mm. or more from the base (Plate 2, figs. 8 and 11). At the apex they are divided into a number of lanceolate lobes, 1·5 to 2·5 mm. in length, or, perhaps, even longer (Plate 2, fig. 8). The preservation of the specimens is not, however, very good in this region, and the details cannot be made out with accuracy. In the clearest examples the "cupule" appears to enclose the seed somewhat loosely, and the lobes at the apex are erect and not spreading.

It is impossible to obtain any evidence as to whether the seeds in these specimens are fully mature. It has been shown that in *L. Lomaxi* only the young seeds are constantly enclosed in a "cupule," and that they are generally naked* when fully developed. The same may have been the rule in *L. Sinclairi,* in which case these seeds would be regarded as not having reached their full maturity. But of this there is at present no conclusive evidence.

---

* Oliver and Scott, *ibid.*, p. 215.

A microscopic examination of these "cupules" has not revealed the existence of any large, prominent capitate glands, similar to those which form so characteristic a feature of the "cupule" of *L. Lomaxi.* But in the case of *L. Sinclairi,* the evidence on this point is of little value. These seeds are preserved merely as structureless casts, and even if the preservation had been better than it is, it would be unlikely that delicate emergences of this type could be recognised supposing they had existed in the living state.

(3) *Position of the Seed on the Plant.*

Another interesting point in connection with these specimens is found in the fact that the great majority of the seeds are still actually attached to the axes on which they were borne in the living state (Plate 2, figs. 7, 8, 10, and 11). Here there can be no doubt as to the continuity of the seed, its "cupule," and the branched axes. The axis appears to have been of a highly-compound nature, and the seeds to have terminated the finer branches (Plate 2, figs. 7 and 10). The evidence of these specimens confirms the conclusion already expressed as the result of the recent investigation of *Lagenostoma Lomaxi,* that the seeds were in all probability not borne in a definite strobilus or cone, but that the fructification was a lax one.†

As to the morphological nature of these axes, two views might conceivably be held. In the first place, it is possible that they may be the ultimate branches of a compound shoot, *i.e.,* of the nature of a stem. Some specimens of *Lyginodendron* have been recently found to have branched repeatedly,‡ and it is, therefore, conceivable that the axes in question may be of this nature.

It is, however, much more probable that the seed-bearing branches are modified fronds in which the lamina has been reduced. The irregular nature of the branching in these specimens, and the fact that some of the seeds terminate short branches, and others are borne at the ends of the longer

---

* Oliver and Scott, *ibid.,* p. 224.

† Oliver and Scott, *ibid.,* p. 229.

‡ Lomax, "On some New Features in Relation to *Lyginodendron Oldhamium,*" 'Ann. of Bot.,' vol. 16, p. 601, 1902.

axes, is readily intelligible on this view, and is unlike anything with which we are acquainted if these axes be really of the nature of shoots. Also, the absence of any indication of foliar organs is remarkable on the latter supposition. Bearing in mind the fact that in the only known case in which we have any evidence as to the manner in which the seeds were borne in the Cycadofilices, that of *Medullosa,* recently discovered by Mr. Kidston, they occur on the normal pinnæ of a frond, the foliar nature of these branches in this instance is also highly probable. This conclusion is supported by the highly-compound nature of the frond attributed to *Lyginodendron* (*Sphenopteris Hœninghausi*), from which, by reduction of the lamina, these branches might well be derived. In the case of Stur's *Calymmatotheca Stangeri,* a fossil recently discussed by Professor Oliver and Dr. Scott,* the fructification is also borne on a frond. Lastly, it has been shown that the anatomical evidence of *Lagenostoma Lomaxi* "clearly indicates that the cupule together with the pedicel was of a foliar nature."† We may, therefore, conclude with every degree of probability that the branched axes on which the seeds, *L. Sinclairi,* were borne are of the nature of segments of a compound frond with reduced lamina, and in view of the close affinity of this seed to that recently attributed to *Lyginodendron,* in addition to the characters of these branched axes themselves, this frond was probably of the *Sphenopteris* type.

Summary.

The following is a brief summary of the morphology of the seed, *Lagenostoma Sinclairi,* Kidston M.S.

The seeds are radiospermic, and elliptical-oblong in shape. They vary from 4 to 5·5 mm. in length, and 1·5 to 3 mm. in breadth. The testa is smooth, and slightly ridged longitudinally.

The integument at the apex is notched or fluted in a manner similar to the apical portions of *Lagenostoma ovoides* and *L. Lomaxi.*

The seed was enclosed in a "cupule," in many respects recalling that of *L. Lomaxi.* The length of the "cupule" is from 8 to 9·5 mm. The "cupule," at the stage of development represented in these specimens, entirely envelopes the seed. The "cupule" was a sac-like organ, prominently ridged longitudinally, and divided at the apex into a number of lanceolate, erect lobes.

The seeds, enclosed in their "cupules," were borne on the terminations of the finer branches of a highly compound frond with reduced lamina, in all probability of the *Sphenopteris* type.

---

* Oliver and Scott, *ibid.*, p. 230.

† Oliver and Scott, *ibid.*, p. 229.

General Conclusions.

As has been already indicated, three species of *Lagenostoma* have been previously described, and these are known only in the petrified condition. Although, in the case of the two new species discussed here, the internal structure of the seed is not preserved, they agree so closely in their general morphology with those already recorded that there can be no hesitation in referring them to the same genus. *L. Kidstoni* approaches nearer to *L. physoides* than to either of the other species, both in point of size, and in the important morphological characters presented by the apical lobes. It is, however, easily distinguished by the smaller number of lobes, as well as by the absence of other external characters peculiar to that species. On the other hand, *L. Sinclairi* appears to agree more closely with *L. ovoides* as to size, and recalls also *L. Lomaxi* in respect to the condition of the integument at the apex of the seed and the presence of a "cupule."

Although numerous casts or impressions of detached seed-like bodies from the Upper Carboniferous rocks of Britain and the Continent have been figured in various memoirs, I am not aware of any which may, without hesitation, be referred to the genus *Lagenostoma.* There are, however, a few specimens described by Continental botanists, which may be mentioned in connection with the species under discussion here.

In 1804, Schlotheim* figured a pinnately branched axis, on which numerous oval bodies, of fairly large size, and conceivably of the nature of impressions or casts of seeds, were attached to the secondary branches. Schlotheim described these bodies as "Blasen" or "Beeren." Potonié† has, however, re-examined Schlotheim's specimen, and has come to the conclusion that the bodies in question are really of the nature of diseased pinnules, due either to insect or fungal agency. Other specimens figured by Gœppert,‡ and Geinitz§ are probably of a similar nature, and there is no satisfactory evidence that the fronds in question, for which Gutbier,|| in 1843, proposed the name *Weissites*, are really the fertile leaves of *Odontopteris.*

There remain, however, some impressions, figured by Geinitz¶ in 1855, and

---

* Schlotheim, 'Ein Beitrag zur Flora der Vorwelt,' p. 58, Plate 13, fig. 26, 1804 ; 'Die Petrefactenkunde,' p. 413, 1820.

† Potonié, "Die Flora der Rothliegenden von Thüringen," 'Abhandl. K. Preuss. geol. Landes,' Neue Folge, Heft 9, Theil 2, p. 32, Plate 2, fig. 1, 1893.

‡ Gœppert, 'Die Gattungen der Fossilen Pflanzen,' Lief 5—6, pp. 98 and 100, Plate 6, 1841.

§ Geinitz and Gutbier, 'Die Versteinerungen des Zechsteingebirges . . . Systems in Sachsen,' vol. 1, p. 21, Plate 8, fig. 8, 1848.

|| Gutbier in Geinitz, 'Gäa von Sachsen,' p. 85, 1843.

¶ Geinitz, 'Die Versteinerungen der Steinkohlenformation in Sachsen,' pp. 21 and 57, Plate 26, figs. 10, 10*a*, and 11, 1855.

regarded, on quite inadequate grounds, as the fructification of *Odontopteris britannica,* which are more worthy of consideration in this connection. We are not here concerned with the question of their attribution, which appears to be still problematical, especially in the light of M. Grand'Eury's recent suggestion* as to the probable nature of the female fructification of this genus, which does not agree at all with the characters exhibited by Geinitz's figures. These specimens consist of a number of small bodies attached to an axis, but their structure is not very clear, judging by the drawings given. I do not regard it as certain that these bodies are really of the nature of seeds, though I admit the possibility of their being of this nature, so far as one can judge without any opportunity of examining the actual specimens. On the other hand, there is an equal probability that these specimens may be of a similar nature to those figured by Schlotheim and Gœppert, to which reference has been made above, and this probability renders unnecessary any further comparison with the Lagenostomas described here.

It has been already pointed out that the presence of a "cupule" in the case of *L. Sinclairi* is a character of special interest. Of the three species previously recorded, only one has been found to have possessed this organ. In *L. physoides* and *L. ovoides,* the seed appears, on the present evidence, to have been naked, a condition similar to that which pertained in the seed described here as *L. Kidstoni* at the stage in its development represented by these specimens. The "cupule" of *L. Sinclairi* probably differed only in details from that of *L. Lomaxi.* It forms, so far as one can judge, a close investment to the seed, and although lobed distally, the undivided portion is considerably longer than the seed itself. A similar condition of affairs existed at certain periods in the development of the seed, *L. Lomaxi.* In *L. Sinclairi,* the apical lobes appear to be erect, but in the living state their disposition was doubtless such as to allow free access for the microspores to the pollen-chamber, a condition which we know to be necessary from the recent exposition of the pollination mechanism in seeds of this type.

A "cupular" investment to the seed is only known, at present, in three instances among the fossils of this period. In *L. Lomaxi* and *L. Sinclairi,* the "cupule" subtends a single seed, whereas in *Gnetopsis elliptica*† as many as four seeds may be enclosed in one "cupule." These are the only recorded examples among Palæozoic plants. While our knowledge of this organ is, therefore, too scanty at present to afford sufficient evidence as to its origin and homologies, it is, perhaps, permissible to enquire whether it may not in some degree have been *analogous* to the carpellary investment of Angiospermous seeds. In the latter case, the functions of the carpels are manifold. Not only do they contribute in a marked degree to the pollination mechanism of the seed, but at least one of their other functions is to serve as protections to the developing ovules. The latter would seem to be the earlier and more primitive of the two, for in these Lagenostomas the protective function of the "cupule" is probably the main service which it performs, although a suspicion is not wanting that even here, where the "cupule" takes no direct part in pollination, it may have been of importance indirectly in furthering the act of fertilisation.* Thus, while offering no suggestion as to homologies, which may or may not exist between these two types of seminal investment—a question which in the present position of our knowledge of these fossil seeds we are hardly warranted in discussing—it can scarcely be doubted that the function of the "cupule" was in part at least that of a protective investment, and that the organ was in this respect analogous to the carpels of the higher plants.

---

* Grand'Eury, "Sur les graines des Neuropterideæ," 'Comptes Rendus, Acad. Sci. vol. 139, p. 25, July 4, 1904.

† Renault, 'Cours Bot. Foss.,' vol. 4, p. 180, Plate 20, 1885.

At the present time, we are acquainted with the manner in which the seeds were borne in the Pteridospermeæ in one instance only. The material on which the correlation of *L. Lomaxi* with *Lyginodendron* was based, did not afford any direct evidence of this nature. But in the specimens recently described by Mr. Kidston, organic continuity existed between the foliar and reproductive organs. It was shown that, in some Medulloseæ, large seeds terminated an ordinary frond of the *Neuropteris* habit, with pinnules in all respects similar to those of the sterile fronds of that genus.

The new specimens discussed here unfortunately yield no direct evidence as to the type of sterile frond associated with these seed-bearing axes; which themselves, apparently, did not bear foliar organs. The presence of detached pinnæ of *Sphenopteris obtusiloba,* which occur with both these seeds (Plate 1, photo. 2), is, in itself, quite without any reliable value as an indication of the character of the foliar organs of the plants to which these seeds belong. But the general morphology of these branched axes indirectly affords some evidence on this point, and in *Lagenostoma Sinclairi,* and possibly also in *L. Kidstoni,* we have the first clue to the habit of the Lyginodendreæ as regards the manner in which the seeds were borne.

In discussing the nature of the seed-bearing axes, it was pointed out (p. 254) that they are best regarded as portions of a highly-compound frond with reduced lamina. In the case of *L. Kidstoni,* although the long rachis-like structures do not afford any conclusive evidence on this point, they nevertheless present many points of morpholcgical similarity to the sterile fronds of the genus *Sphenopteris*. In *L. Sinclairi*, the most reasonable interpretation of the irregularly branched axes is that of a highly-compound frond, which, had it possessed a lamina, would in all probability be placed in the form-genus *Sphenopteris*.

---

* Oliver and Scott, *ibid.*, footnote, pp. 214, 215.

Thus, in both cases, the evidence such as it is, agrees with the provisional conclusion that the sterile foliage associated with these seed-bearing axes was probably of the *Sphenopteris* type.

This conclusion is supported by the recent attribution of the seed *L. Lomaxi* to *Lyginodendron*, a stem known beyond doubt to have possessed fronds of this nature. There is therefore reason to suppose that these new species which, in the morphology of their seed-bearing axes, recall the foliar organs of *Lyginodendron*, and which in the nature of the seeds agree so well with *L. Lomaxi*, were borne by stems either of the genus *Lyginodendron* itself or of some other closely related member of the same family, possessing, in all probability, the *Sphenopteris* form of sterile foliage. In *L. Sinclairi*, the presence of a "cupule"—a rare occurrence among Palæozoic seeds—not dissimilar to that which has been described in the case of *L. Lomaxi*, lends additional support to this argument.

We have thus in these specimens the first definite clue to the habit of the Lyginodendreæ as regards the female fructification, a character in which some of them, at least, differed from the Medulloseæ. We may picture them as plants which, in addition to the numerous, highly-compound fronds of the *Sphenopteris* type, bore others in which the lamina were wholly or partly reduced, the ultimate branches terminating in seeds with, or without a "cupular" investment.

In the lax arrangement of the fructification, the Pteridospermæ must have presented a striking contrast in habit to the members of most of the other great Palæozoic groups, in which the compact strobili were, for the most part, conspicuous and dominant types of sporangial aggregation. Among living plants almost the only analogue is to be found in the female sporophyll of *Cycas*.

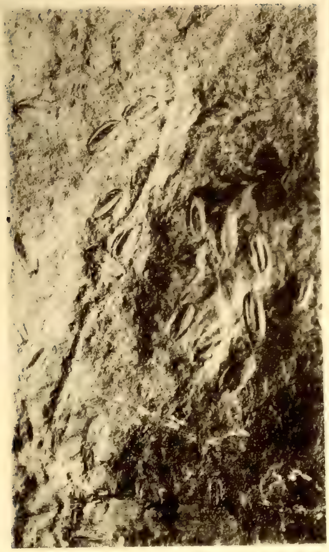
EXPLANATION OF THE PLATES.

Plate 1.

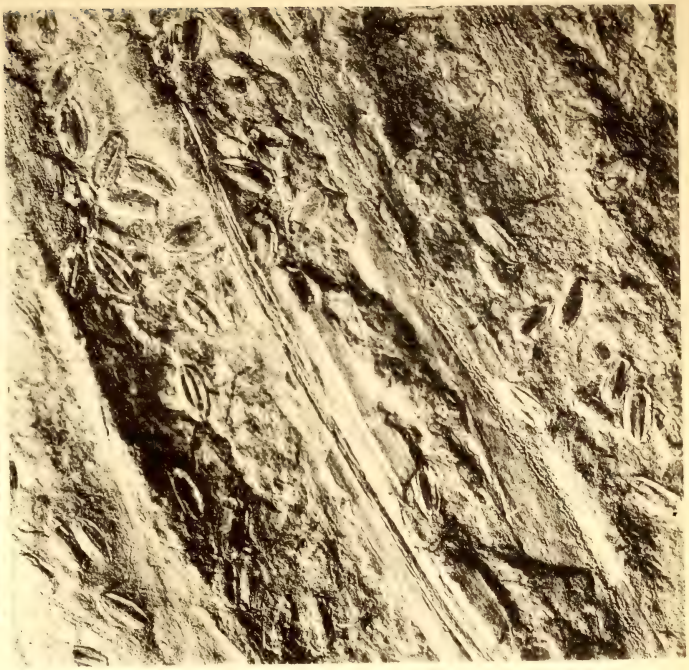
Lagenostoma Kidstoni, sp. nova.

(Photos. 1, 3 and 4 by Mr. L. A. Boodle ; photo. 2 by Mr. H. G. Herring.)

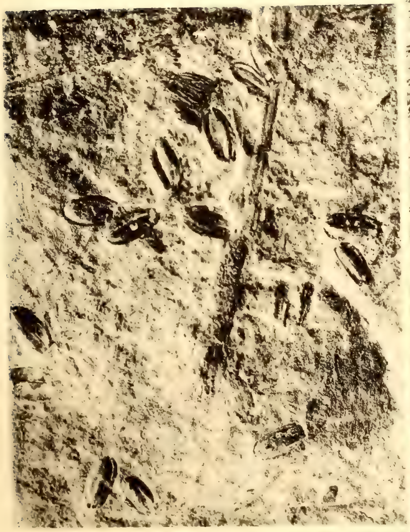
Photo. 1.—A portion of the Glasgow specimen, showing the numerous seeds scattered over the surface of the shale. The longitudinal ridges and the apical lobes are clearly seen in many instances. Three of the long rachis-like structures associated with the seeds are shown. × $\frac{4}{3}$.



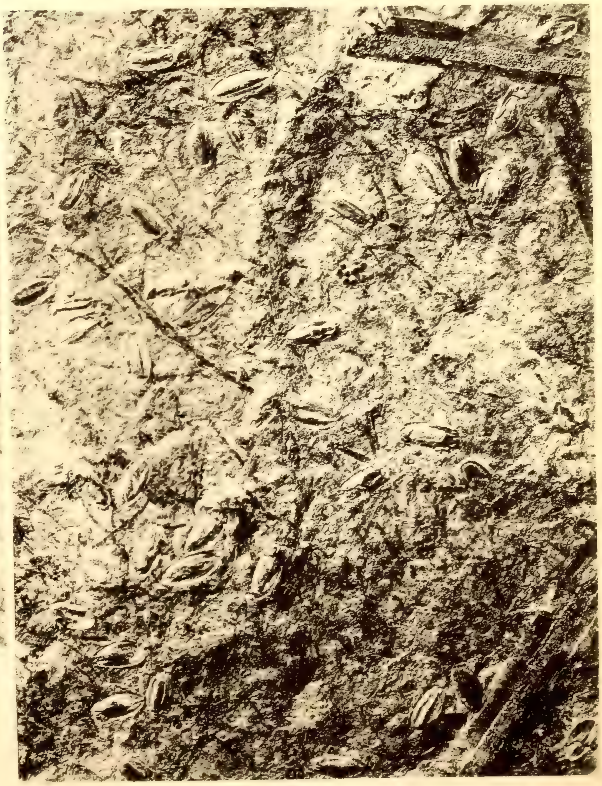
2



1 $\times \frac{4}{3}$



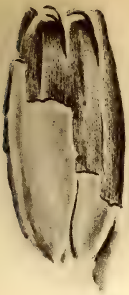
4 $\times \frac{4}{3}$



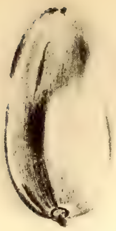
3 $\times \frac{4}{3}$

PHOTOS. BY L. A. BOODLE & H. G. HERRING,

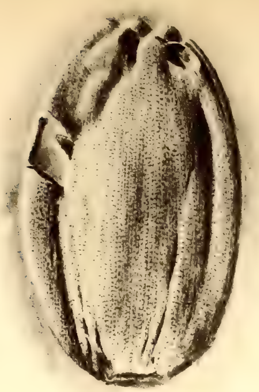
LAGENOSTOMA KIDSTONI, sp. nova.



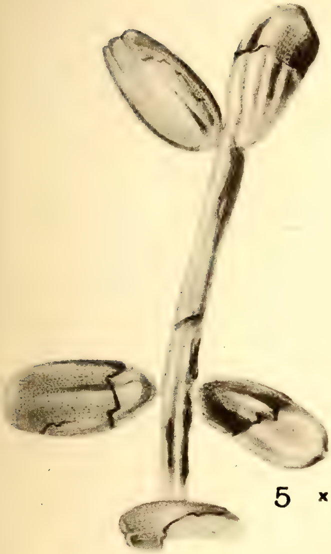
3 ×5



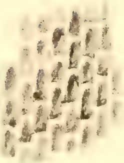
2 ×5



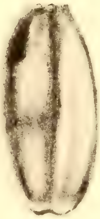
1 ×8



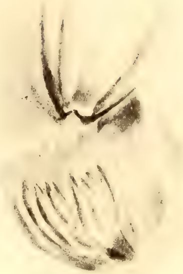
5 ×3



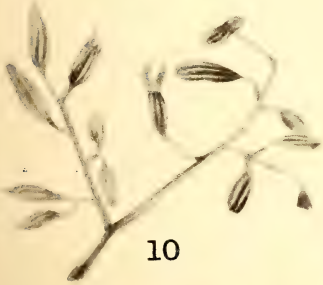
6 ×70



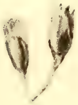
9 ×5



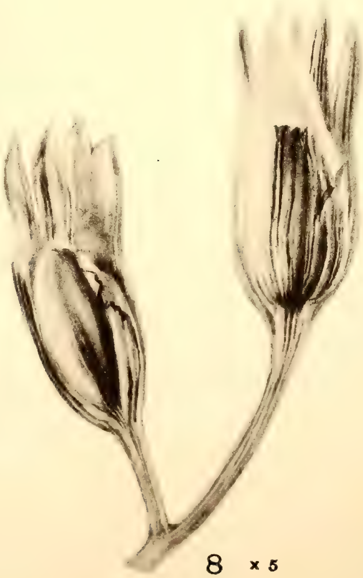
4 ×3



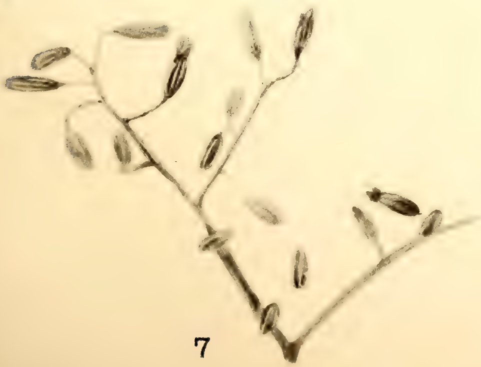
10



11



8 ×5



7

DEL. J. ALLEN & G. M. WOODWARD.

LAGENOSTOMA KIDSTONI sp. n. & L. SINCLAIRI, Kidston m.s.

Photo. 2.—A portion of the British Museum specimen, showing several seeds. A pinnule of *Sphenopteris obtusiloba* is also seen. Nat. size.

Photo. 3.—A portion of the Glasgow specimen, showing numerous well-preserved seeds and portions of the rachis-like structures. The apical lobes of several of the seeds are clearly seen. × $\frac{4}{3}$.

Photo. 4.—A portion of the Glasgow specimen, showing some well-preserved seeds. A multilobed foliar structure, possibly of the nature of a "cupule," is seen in the neighbourhood of one of the seeds, near the top of the photograph. × $\frac{4}{3}$.

PLATE 2.

Lagenostoma Kidstoni, sp. nova, and *L. Sinclairi*, Kidston M.S.

(Drawings 1—4 and 6 by Mr. J. Allen ; drawings 5 and 7—11 by Miss G. M. Woodward.)

FIG. 1.—*Lagenostoma Kidstoni.*—A seed from the Glasgow specimen, showing five of the apical lobes. × 8.

FIG. 2.—*L. Kidstoni.*—A seed from the Glasgow specimen, showing the hilum. × 5.

FIG. 3.—*L. Kidstoni.*—A seed from the Glasgow specimen, showing six apical lobes. The integument, represented by a carbonaceous layer, is only preserved in the upper portion of the seed. × 5.

FIG. 4.—*L. Kidstoni.*—The basal portion of a seed from the Glasgow specimen. In the neighbourhood of the seed a sheath-like body, possibly of the nature of a "cupule," and divided into a number of teeth at the apex, is somewhat indistinctly seen. The same body is also seen in Plate 1, photo. 4. × 3.

FIG. 5.—*L. Kidstoni.*—Several seeds, apparently in continuity with an axis. The integument of the seeds is only partly preserved. From the British Museum specimen. × 3.

FIG. 6.—*L. Kidstoni.*—Highly magnified cells of the integument of a seed shown on the Glasgow specimen. × about 70.

FIG. 7.—*L. Sinclairi.*—A highly branched axis, the branches being terminated by seeds, in most cases enclosed in a "cupule." Kidston Coll. No. 3530. Nat. size.

FIG. 8.—*L. Sinclairi.*—An enlarged drawing of two seeds enclosed in their "cupules," and borne on a branched axis. Kidston Coll. No. 3531. × 5.

FIG. 9.—*L. Sinclairi.*—An enlarged drawing of a seed without its "cupule," showing the flutings at the apex. Kidston Coll. No. 3530. × 5.

FIG. 10.—*L. Sinclairi.*—A number of seeds, most of which are enclosed in "cupules," and attached to the finer branches of a compound frond with reduced lamina. The ribbed nature of the "cupule" is clearly seen. Kidston Coll. No. 3529. Nat. size.

FIG. 11.—*L. Sinclairi.*—Two seeds enclosed in their "cupules," of which an enlarged drawing is shown in fig. 8. Kidston Coll. No. 3531. Nat. size.

Ovulation and Degeneration of Ova in the Rabbit.

By Walter Heape, M.A., Trinity College, Cambridge.

(Communicated by Adam Sedgwick, F.R.S. Received March 6,—Read April 6, 1905.)

It has long been held that ovulation invariably occurs in all animals at each period of œstrus.

I have already shown (Nos. 13, 14, 15) that this is not necessarily true for menstruating animals. In other polyœstrous animals also there seems reason to believe that when coition is prevented during the first few recurrent œstrous periods, ovulation is interfered with during the subsequent periods, for conception is then much more uncertain than it is if coition occurs when the sexual season first appears.

Moreover, among bats there is clear evidence that ovulation does not necessarily occur during œstrus (Nos. 2, 4, 5, 8), for the mature females are impregnated in the autumn and do not ovulate until later, probably the following spring; although the young females, born in the late spring, do not copulate until the spring of the following year at the time when ovulation also occurs.

With these facts before me I began, in 1894, investigations on the domestic rabbit. Over one hundred does were experimented on, and being kept in locked cages, of which I only had the key, certain errors so common with breeding experiments were avoided. The ovaries were preserved in various ways, and the histological results here given are determined from serial sections of which I have some 120 series.

It was found that the domestic rabbit does not ovulate until, approximately, 10 hours after copulation (*cf.* Nos. 1, 3). The doe rabbit only permits coition when undergoing œstrus, and if the male is withheld at that time the ripe ova in the ovary degenerate; they are not dehisced from the ovary. Neither stimulation of the vulva with electrodes, nor artificial insemination, nor subcutaneous injection of spermatozoa induced ovulation; moreover ovulation did not follow coition if from any cause a sufficient supply of blood to the ovaries was interfered with; while at the same time, provided this supply of blood was not interfered with, artificial stoppage of the progress of the spermatozoa from the vagina did not interfere with ovulation.

The Graafian Follicle and Ovum.—The follicle consists of a thick layer of epithelium, bounded on its outer edge by a basement membrane. Within, the ovum lies surrounded by its zona radiata. The structure is embedded in the ovarian tissue, which consists essentially of a connective tissue network enclosing large parenchyma cells, through which blood vessels run. In the region of the follicle this tissue is somewhat modified. Close round the basement membrane I find a thin layer of parenchyma cells in a connective tissue network, the theca interna, and immediately outside that the connective tissue itself is specially developed into closely approximated fibrous bands with a few parenchyma cells in the interstices thereof—the theca externa (Nos. 23, 29, 30, 33).

The first sexual season begins in the domestic rabbit as a rule in February or March, but is dependent somewhat on the food given, on the warmth and shelter provided, and on the frequency with which they were allowed to produce young the previous year.

Prior to that time certain of the Graafian vesicles enlarge, a space containing liquor folliculi appears, and the ovum, surrounded by a thickened mass of epithelial cells (the discus proligerus) lies eccentrically but connected with various portions of the follicle wall by strands of this tissue. The remainder of the epithelium round the wall of the follicle is much reduced in thickness. The growth of those vesicles situated near the surface of the ovary causes them to project and form swellings on the surface; both the wall of the follicle and the tunic of the ovary is here very much attenuated, so much so that in some of them, when ripe, the structure is sufficiently transparent to allow of the ovum being seen within the vesicle. During procœstrum, the blood vessels which surround these follicles become more numerous, enlarged and congested, and such as run in the thin wall which projects on the surface of the ovary give these follicles the bright pink colour which is characteristic of them.

It is important to notice, however, that a brilliant suffused red colour does not denote a ripe Graafian vesicle but, as I will show below, a degenerate follicle which will not rupture; failure to distinguish between these two appearances has been the cause of much error.

Maturation of the Ovum.—Normally, immediately after copulation the ripe ovum in the swollen Graafian follicle is affected. The cells of the discus proligerus, which until now had closely invested the ovum, begin slowly to withdraw (*cf.* 19). During the growth of the ovum nutriment is supplied to it by the aid of these cells; as I have already shown (No. 12), protoplasmic processes from them are projected into the radiating canals of the zona radiata; now the cells withdraw radially and eventually remain attached to the zona only by these exceedingly fine strands. This process apparently occupies some hours. At the same time the ovum, bounded by its thin vitelline membrane, withdraws somewhat from the zona leaving a narrow space between them. It appears obvious that thus the supply of ovarian nutriment is stopped. About nine hours after copulation maturation of the ovum takes place and two polar bodies are rapidly formed.

It is of interest to notice that maturation does not occur until after the supply of nutriment to the ovum is cut off, for in this particular it appears probable that the formation of polar bodies takes place under conditions of nutriment essentially different from those which prevail during segmentation.

Ovulation.—About 10 hours after copulation the Graafian vesicle ruptures through the attenuated wall which projects on the surface of the ovary. The ovum, entirely freed from the discus proligerus cells, is shot out into the infundibulum which now closely invests the ovary.

Once freed from the ovary the mature ovum is incapable of assimilating nutriment unless it be fertilised; if from any cause fertilisation is not effected the ovum quickly dies, although it is bathed in the nutrient material supplied by the maternal tissues; ova thus degenerating are, from time to time, to be seen in the fallopian tubes.

It is necessary then, in the case of rabbits at any rate, that spermatozoa should be present in the fallopian tubes, and I find that as a rule they are to be found at the top of the uterus horn two hours after copulation and close to the infundibulum, if not actually within its folds, four hours after copulation.

In those animals in which the ovum does not undergo maturation in the ovary, the presence of spermatozoa at the top of the fallopian tube is not necessary. In the mouse, for instance (No. 29), when the ovum is dehisced from the ovary it is not free from discus cells, and the polar body may be formed during its passage down the fallopian tube.

Recently it has been shown (No. 23) that in the ferret ovulation does not take place without previous copulation, the same is also said of the pig and sheep (No. 11, *cf.* also, No. 22) and guinea-pig (No. 26), and I suspect it will also be found to be true for other animals. It is interesting to note that ovulation in the frog takes place only when a certain stage of maturation is reached (No. 24). The fact that maturation of the ovum does not occur until after copulation has taken place affects fundamentally the results of various experiments which have been made on artificial fertilisation of rabbit's ova (Nos. 10, 25, 28).

The Rupture of the Follicle and the Corpus Luteum.—The cause which induces the rupture of the Graafian vesicle is obscure. Immediately before rupture the wall of the distended follicle where it projects on the surface of the ovary is very thin, and is covered by a thin layer of the tunic of the ovary. The congested vessels which surround the follicle are present also between the follicle and the tunic of the ovary in this distended portion.

It has been argued that the follicle ruptures on account of the tension caused by the material secreted therein; but in the rabbit the follicles do not rupture unless copulation takes place and, as I will show below, the ripe follicles which do not rupture because copulation has not taken place are distended and vascular, so far as I can see, to the same extent as those which do rupture.

It has been suggested that the spermatozoa in the infundibulum and, as that organ is closely applied to the ovary, on the surface of the follicle, exercise influence; but as has been shown in the sheep, for instance (No. 22), ovulation may take place prior to copulation, and hence when no spermatozoa are present.

Again, it has been stated that the vessels surrounding the follicle burst and pour their blood into it, so causing increased pressure, which bursts the thin wall; but in the rabbit, at any rate, the blood is not poured in any considerable quantity into the follicles which burst while it floods those which do not burst.

The fact that in the domestic rabbit ovulation does not occur until after copulation has taken place, while in other animals it may occur prior to the sexual act, suggests the probability that in the former animal additional stimulus is necessary to induce the rupture of the follicle. Whether, as has been suggested (No. 27), the rupture is due to the stimulation of erectile tissue or not my experiments do not show, but the observations made on the nerves of the ovary render such explanation extremely probable (Nos. 9, 17, 18, 20, 21, and 34). All I can say is that the base of a discharged follicle appears to be pinched together, though I have been unable satisfactorily to demonstrate that the effect is produced by the contraction of the tissues which surround the follicle, and the actual cause of rupture I have been unable to discover.

The corpus luteum is formed by the ingrowth of cells surrounding the follicle together with the follicular epithelium; the ingrowth being at one time apparently a forcible rush before which the loosened epithelium is driven. The ingrowth takes place in the first instance in the region of the base of the follicle.

Degeneration of Ripe Follicles and the False Corpus Luteum.—When a doe has not been allowed access to the buck during œstrus, the ripe follicles which are present at that time do not burst, and the ripe ova contained therein do not undergo maturation.

The follicle is distended and projects on the surface of the ovary, its outer wall is thin and the whole structure is very vascular, precisely as is the case with the follicles which do rupture after copulation; but it does not rupture, instead, the surrounding congested vessels rupture and pour their blood into the follicle itself, forming there a clot of blood, in the midst of which for many days the degenerating ovum may be seen.

This result causes the brilliant suffused red colour of degenerate ripe follicles at an early stage of the process; gradually the red colour is lost and results in a black patch which long persists, and which is reduced as time goes on by the absorption of the contents of the follicle.

The first rush of blood isolates the ovum and its discus proligerus, and subsequently washes away the rest of the epithelium from the walls of the follicle, disintegrates the theca interna, and permeates the meshes of the theca externa. The contents of such follicles are very brittle, and in sections are frequently lost, so that not infrequently this false corpus luteum appears as a cavity bounded by the theca externa (*cf.* No. 23).

The absorption of the contents is carried out mainly by in-growing parenchyma cells, though a few leucocytes are also similarly engaged, and as the blood clot disappears its place is taken by the normal ovarian tissue. Thus, the false and the true corpora lutea are markedly different structures and are readily distinguishable in sections.

Degeneration of Ova and Sterility.—If the buck is withheld from a doe during several consecutive periods of œstrus, most, if not all, the older and many younger follicles then undergo degeneration. The loss of ova from this cause is so great that frequently during the remainder of that breeding season, and sometimes apparently for one or more future seasons, the animal is sterile. This prevention for a time of the normal functions of the ovary results in more or less persistent sterility, a point of interest to students of the physiology of the generative system and not without economic importance.

Degeneration of Young Follicles: Variation and Nutrition.—But besides degeneration brought about in this manner, there are other causes which induce degeneration of ova. In many ovaries in which there are healthy ripening ova in healthy follicles, degenerative changes are to be seen in others, in some of which it is the follicle, in others the ovum, which first shows signs of disintegration. These are invariably younger follicles, and the cause of degeneration seems clearly to be associated with nutrition.

In cases where *follicles* degenerate in the neighbourhood of other healthy follicles, want of nutrition is strongly indicated, and it is not improbable that competition is at work and is responsible for the loss. Degeneration of the *ovum*, however, from the same cause, while it may be due to competition, may also be due either to want of vitality of the ovum or to want of the requisite quality of nutriment, in other words to inability of these ova to assimilate what is obviously sufficient for the needs of neighbouring ova. In the latter case the constitutional capacity of the ova is concerned, and while their degeneration may be due to a defective supply of nutriment, it may, with no less probability, be inferred that failure to develop is due to their incapacity to utilise the nutriment which is supplied and on which other neighbouring ova flourish.

It is well known that domestic animals and cultivated plants are more prone to vary than are the same varieties or species in a wild state. De Vries (No. 32) expresses the view that by feeding up the mother-plant with manure the offspring are induced to exhibit a greater variation; that by rich or poor treatment of seed-plants greater variation can be produced than by selection of seeds; and that the influence of manures on the mother-plant is exercised on the seeds she produces. If this be so, any increased capacity on the part of the mother to assimilate nutriment of different qualities, or to manufacture such material, will enable her to produce more widely varying offspring.

I would suggest then that when young ova degenerate under the conditions above specified, there is great probability that in such cases the degeneration is due to peculiarities in the constitution of those ova, and that such ova require special facilities for development; that they give rise to, in fact, "sports," extreme cases of that variation which it is known domesticated animals are specially liable to produce. In view of De Vries' observations and the experience of many practical breeders and horticulturists, it would seem very important that the whole question of the effect of various kinds of nutriment upon the developing ovum and embryo should be investigated, for it is reasonable to expect that, given the requisite quality of nutriment, the power of producing variable offspring would be widely extended and the field for the study of variation correspondingly enlarged.

The Ovary as a Secretory Gland.—Evidence of the part the ovary takes in providing the ovum with nutriment demonstrates that it is a secretory gland, and it has been urged that ovarian secretion is responsible for much besides the growth of the ovum, that it governs, indeed, all activity of the other generative organs.

The experience of ovariotomists shows that excision of the ovaries has a marked effect on procœstrum (menstruation—human) and upon the severity of œstrus (mares), and it is claimed, though it appears to me this requires confirmation, that destruction of the corpora lutea prevents the gestation of the ova which were discharged from the pre-existing Graafian vesicles (*cf.* Nos. 6 and 7).

These facts would certainly indicate that if the force which controls the activity of the whole generative system is not in the ovary, that organ is at least essential to the normal functions of the remainder of the system. In the same way excision of the generative glands affects, for instance, the growth of horns in the male, and exercises influence in various ways on other organs of the body of both sexes. Thus these organs are probably essential to the normal development of sexual characteristics.

There would seem to be little room for doubt that this is the case. But in all animals which have a special breeding season, the ovary has a quiescent period, so far as the development of the ova are concerned, varying in accordance with the length of the interbreeding seasons. For some time before the advent of the sexual season, however, with the probable exception of bats, the ovary exhibits activity, and is obviously engaged in transmitting nutriment to the ova, which now begin to develop. The commencement of this active season is marked by the increased vascularity of the gland. The fact that this ovarian activity precedes the sexual season is not unfavourable to the view that it exercises influence on the latter. It must be recollected, however, that monkeys, although they menstruate regularly each month throughout the year, have a subscribed breeding season, and their regular menstrual function goes on during the time when the ovary is quiescent. Moreover, as ovulation in the bat may take place many months after the sexual season is over, and the bat's ovary is certainly quiescent during those intervening months, it may be expected that the sexual season in bats occurs in the absence of ovarian activity. Finally, it is quite clear that ovulation and pr.œstrum are not necessarily coincident in many animals.

If these facts are true, it does not seem possible to accept the view that the stimulus which induces proœstrum and œstrus has its origin in the ovary.

There is another point to be considered, namely, that the advent of sexual activity may be hastened or delayed or, perhaps, prevented altogether; the severity of proœstrum and œstrus augmented or reduced; and the ratio of fertility influenced, by climatic conditions and food.

Similar conditions obtain and similar results follow in the male.

Generative Ferment and "Gonadin."—My belief is that the stimulus which primarily induces such activity is of extraneous origin; that it is due to a change in the constitution of the blood, brought about by climatic influences and food, which from the nature of its growth would seem to be always specially nutritious at this season; that it results in increased vitality throughout the body—clearly evidenced by the growth of horns, wattles, and other excrescences, by the growth of hair and plumage, and the accession of brilliant colouring to such epidermic growths or to the skin itself—and similarly affects the generative system.

The substance which causes this change in the constitution of the blood, which creates disturbance and results in activity, is probably of the nature of a ferment. It affects similarly both male and female animals, and may be considered as a special "generative ferment."*

As I have already shown, there is strong evidence that the increased activity of the generative glands, consequent on the presence of this "ferment," results in the secretion of material which exercises a profound effect upon the rest of the generative system and possibly upon other organs; for this secretion I will suggest the term "gonadin" should be used.

LITERATURE.

1. Barry, "Researches in Embryology," 'Phil. Trans.,' 1839.
2. Benecke, "Ueber Reifung und Befruchtung des Eies bei den Fledermäusen," 'Zoolog. Anzeiger,' vol. 2, 1879.
3. Beneden, "Recherches sur l'Embryologie des Mammifères," 'Archives de Biol.' vol. 1, 1880.
4. Beneden and Julin, "Observations sur la Maturation, la Fécondation, etc., chez les Cheiroptères,' 'Archives de Biol.,' vol. 1, 1880.
5. Eimer, "Ueber die Fortpflanzung der Fledermäuse," 'Zoolog. Anzeiger,' vol. 2, 1879.
6. Fraenkel and Cohn, "Experimentelle Untersuchung ueber den Einfluss des Corpus Luteum auf die Insertion des Eies," 'Anat. Anzeiger,' vol. 20, 1901.
7. Fraenkel, "Die Function des Corpus Luteum," 'Archiv f. Gynækol.,' vol. 68, 1903.
8. Fries, "Ueber die Fortpflanzung der Einheimischen Cheiropteren," 'Göttinger Nachrichten,' 1879.
9. Gawronsky, "Ueber Verbreitung und Endigungen der Nerven in den weiblichen Genitalien," 'Archiv f. Gynækol.,' vol. 47, 1894.
10. Grusden, "Versuche über die Künstliche Befruchtung von Kaninchen Eiern.," 'Archiv f. Anat. und Physiol. Anat. Abtheil.,' 1896.
11. Hausmann, "Ueber die Zeugung und Entstehung des Wahren Weiblichen Eies bei den Säugethiere," 1840.
12. Heape, "The Development of the Mole. The Ovarian Ovum," 'Quart. Jourl. of Microscop. Science,' vol. 26, 1886.
13. Heape, "The Menstruation of Semnopithecus Entellus," 'Phil. Trans.,' 1894.
14. Heape, "The Menstruation and Ovulation of *Macacus rhesus*," 'Phil. Trans., 1897.
15. Heape, "The Menstruation of Monkeys and the Human Female," 'Trans. Obstet. Society,' vol. 40, 1898.
16. Heape, "The Sexual Season of Mammals," 'Quart. Jourl. of Microscop. Science,' vol. 44, 1900.
17. Herff, "Ueber den feineren Verlauf der Nerven im Eierstock des Menschen," 'Zeit. f. Geburt. und Gynækol.,' vol. 24, 1892.

---

* I originally suggested "œstrus toxin" as a name for this disturbing agent, but, as its influence is not confined to the female, and as there is no reason to assume the presence of poison, this term is misleading.

18. Herff, "Giebt es ein Sympathisches Ganglion im Menschlichen Ovarium," 'Archiv f. Gynækol.,' vol. 51, 1896.
19. Holl, "Ueber die Reifung der Eizelle bei den Säugethieren," 'Sitz. der Kaiser. Akad. der Wiss.,' Wien, vol. 102, 1893.
20. Kallius, "Nervendigungen in Drüsen. 1. Eierstöcke," 'Ergebnisse der Anat. und Entwicklungsgeschichte,' vol. 4, 1894.
21. Mandl, "Ueber Anordnung und Endigungsweise der Nerven im Ovarium," 'Archiv für Gynækol.,' vol. 48, 1894.
22. Marshall, "The Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep," 'Phil. Trans.,' B, 1903.
23. Marshall, "The Œstrous Cycle in the Common Ferret," 'Quart. Jourl. of Microscop. Science,' vol. 48, 1904.
24. Morgan, "The Development of the Frog's Egg," 1897.
25. Ott, "Ueberkunstliche Befruchtung durch die Peritonealhöhle," 'Centralbl. fur Gynaekol.,' No. 36, 1882.
26. Reichert, "Beiträge zur Entwickelungsgeschichte des Meerschweinschens," 'Abhandl. der König. Akad. du Wiss. zu Berlin,' 1861.
27. Rouget, "Recherches sur les organes érectiles de la femme," 'Jourl. de Physiol. Brown Sequard,' vol. 1, 1858.
28. Schenk, "Das Säugethierei künstlich befruchtet ausserhalb des Mutterthieres," 'Mitth. aus dem Embryol. Instit.,' Wien, vol. 1, 1880.
29. Sobotta, "Ueber die Bildung des Corpus Luteum bei der Maus," 'Archiv f. Mikr. Anat.,' vol. 47, 1896.
30. Sobotta, "Ueber die Bildung des Corpus Luteum beim Kaninchen," 'Merkel und Bonnet's Jourl. Anat.,' 1897.
31. Stratz, "Der Geschlechtsreife Säugethiereierstock," 1898.
32. de Vries, "Mutationstheorie," 1901.
33. Waldeyer, "Eierstock und Ei," 1870.
34. Winterhalten, "Ein Sympathisches Ganglion im Menschlichen Ovarium" 'Arch. f. Gynækol.,' vol. 51, 1896.

On Reciprocal Innervation of Antagonistic Muscles.—Eighth Note.

By C. S. Sherrington, D.Sc., F.R.S.

(Received April 17,—Read May 18, 1905.)

(From the Physiology Laboratory, University of Liverpool.)

The following note deals more especially with observations on inhibition occurring in instances of "reciprocal innervation" obtained as a spinal reflex reaction. My view is that inhibition of this kind is part and parcel of the normal reflex process, so that in a reflex it goes on side by side with excitation of other muscles opposed to those which are inhibited.* One main consideration which supported the view is the correspondence of the skin-fields whence the reflex contraction of the one set of muscles and the inhibition of the opposed set of muscles can be elicited. So, also, the correspondence of the afferent nerve-trunks, and of the points of surface of the central nervous system whence are elicited the two effects. But to test the view further, I have now attempted to examine in some particulars the conditions attaching to the initiation, and the course run by the two phenomena under comparable circumstances.

I. Even in one and the same spinal region the modes of origination, time-relations, etc., of the several types of reflex elicitable, *e.g.*, in the dog's hind limb, the "extensor thrust," the "direct-flexion reflex," the "scratch reflex," differ so greatly for each of the types as compared with the others, that in order to compare the inhibition phenomenon with the excitation phenomenon it is important to take both the phenomena from the same type-reflex. The type-reflex I have taken for the purpose has been the "direct-flexion reflex" of the hind limb.

Each such type-reflex has features characteristic of it. A salient feature of this reflex chosen is flexion at the knee. For comparison of the inhibition and excitation respectively both hind limbs are taken and so prepared that in one leg only the knee flexors can act, in the other leg only the knee extensors. The stimuli to provoke the reflex are applied either to symmetrical skin-points or to symmetrical afferent nerves at, as far as practicable, symmetrical places in their course. For comparison the stimuli are made as far as possible equal on the two sides. This being arranged certain characteristic features of the reflex have been examined on the two sides respectively.

(*α*) The "flexion reflex" has a "receptive skin-field" which though extensive is characteristic for it. Examined by the above preparation the skin-field whence the excitation (contraction) is elicitable and that whence the inhibition is elicitable has proved in my observations to be one and the same. Thus: stigmatic unipolar faradisation of a point in the skin of a right pedal digit provokes in the homonymous limb contraction of the flexors of the knee, and similar stimulation of the corresponding left digit provokes in its own limb inhibition of the extensors of the knee. Again, similar stimulation of the skin of the fore-foot (in my experience that of the crossed fore-foot acts more readily than that of the homonymous) induces excitation (contraction) of the flexors of the crossed knee; and the corresponding skin-region of the opposite fore-limb induces inhibition (relaxation) of the extensors of knee contralateral to it.

---

* 'Roy. Soc. Proc.,' vol. 52, p. 556, 1893.

(β) Turning to stimuli other than electrical it is not, as I have pointed out, *every* form of stimulus that, when applied within the skin-field appropriate for the "direct-flexion reflex," can excite it. The kinds of skin-stimuli which excite it are those which may be termed "nocuous,"* *e.g.*, a prick, strong squeeze, harmful heat (the heat-beam), chemical agents. Touches, innocuous pressures, rubbing, etc., though effective for various reflexes, *e.g.*, for the "extensor thrust," "scratch reflex," "pinna reflex," etc., do not in my experience excite this reflex. The stimuli which do excite it, for instance, from the *planta*, excite, when applied on the side where the flexor muscles alone remain intact, contraction of those muscles, and when applied correspondingly on the opposite side, where the extensors alone remain intact, inhibit them (relaxation).

(γ) The nerve-twig, similar to that which under faradisation on the "flexors" side excites the flexors (contraction) when faradised on the "extensors" side inhibits the extensors (relaxation). This comparison has been made not only with skin-nerves, but with muscular nerves, notably with the nerves of the hamstring muscles and of the gastrocnemius.

(δ) The "flexion reflex," although it exhibits well the potency of summation of successive stimuli as a factor in its initiation, differs in my experience from various other reflexes,† *e.g.*, "extensor thrust," "scratch reflex," "pinna reflex," in being elicitable fairly easily by a single induction shock. The shock may be applied either to the skin in the receptive skin-field of the reflex or to an appropriate afferent nerve either cutaneous or muscular. When this is done in the prepared limbs the single induction shock applied on the "flexors" side excites a brief reflex contraction of those muscles, correspondingly applied on the "extensors" side it provokes a brief reflex inhibition of those muscles.

---

* Sherrington, 'Journ. of Physiol.,' vol. 30, p. 39, 1903.

† *Cf.* Stirling, 'Arbeiten a. d. Physiol. Anstalt z. Leipzig,' 1874.

(ϵ) The "flexion reflex," unlike "extensor thrust," "pinna reflex," etc., can be well evoked in my experience by make or break of a galvanic current. This make or break reflex is shown in the "extensor" preparation by inhibition, just as it is shown in the "flexor" preparation by contraction. With suitable strength of stimulus the break of a descending current is more effective for the reflex inhibition than the make, and *vice versâ* for an ascending current, just as with contraction. The "flexion reflex" can also to a much greater extent than can the scratch reflex be maintained by passage of the constant current. In this respect it resembles the vasomotor and respiratory reflexes examined by Grützner* and by Langendorff and Oldag,† and also the sensual reaction which similar stimulation excites in ourselves—a point of interest when the connection between *nociceptive* reflexes and dolorous sensation is remembered. When the constant current is thus applied to the limb in which the extensors have been prepared, inhibition proceeds in them as does contraction in the flexors when that current is similarly applied to the limb in which the flexors have been prepared.

(ζ) The latent time of the "flexion reflex" is throughout my experience shorter than that of some other reflexes of the limb, notably than that of the "scratch reflex." This feature is revealed in the inhibition of the extensors just as in the contraction of the flexors. Great differences of latency in the "flexion reflex" as in other reflexes can be obtained by, apart from variance in intrinsic condition of the reflex preparation, variance in the external stimuli in intensity, suddenness, frequency of repetition, etc. The effect of such variations is the same in kind, and, in my experience, in extent, when tested by the reflex inhibition as when tested by the reflex contraction. Thus with strong stimuli I have found as short a latency as 32 σ for the inhibition, which is slightly shorter than the shortest for contraction under like circumstances that I have yet met with. With weak stimuli I have occasionally met with a latency as long as 400 σ for each effect.

(η) A good criterion of comparison between the reflex inhibition and the reflex contraction in the "flexion reflex" under excitation by an intermittent stimulus is the number of stimuli summed for initiation of the reflex as exhibited on the one hand in contraction of the flexors, on the other hand in relaxation of the extensors. The number of successive single stimuli summed for the initiation is less as their individual intensity is greater.‡

When the summation is compared, in the same reflex preparation, in the reflex exhibited as inhibition (relaxation) in the knee extensors of one limb

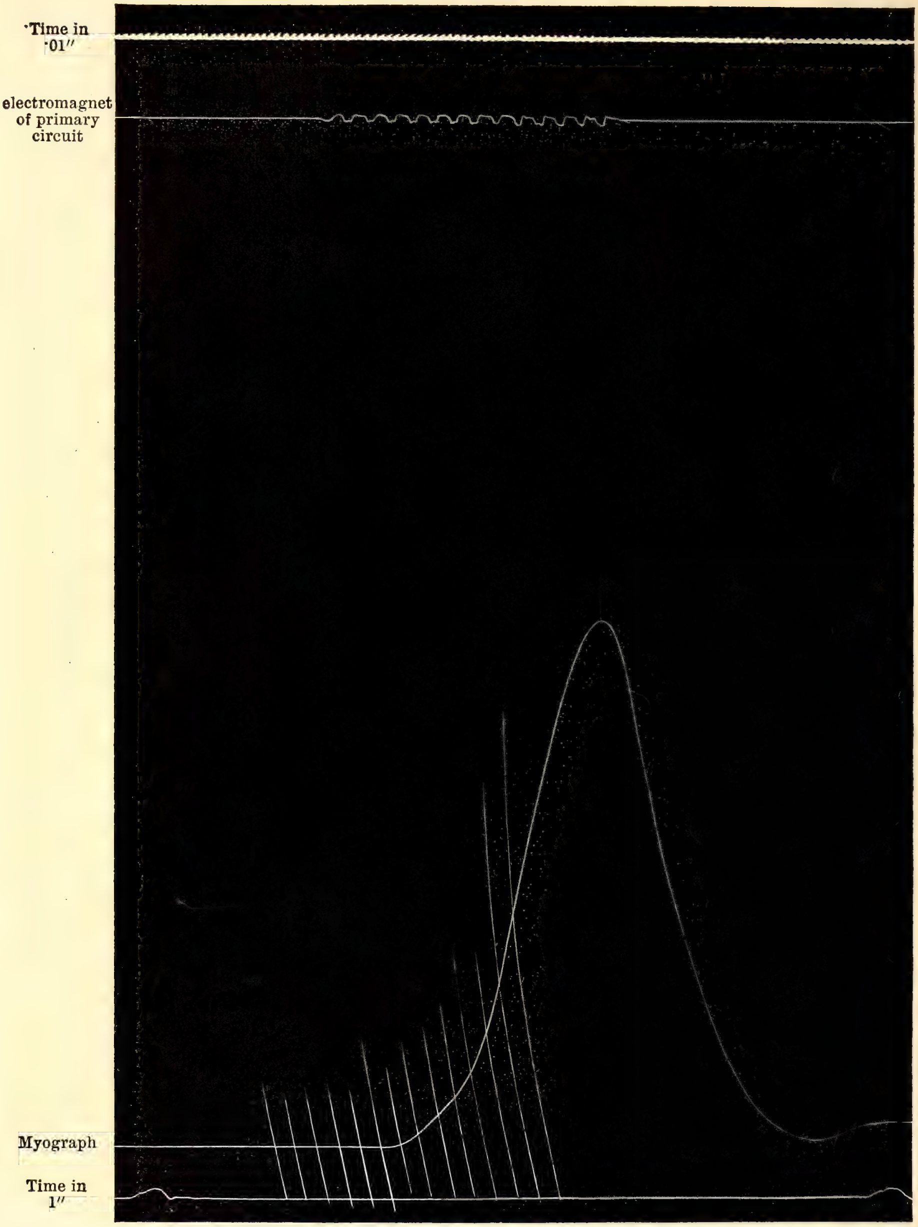
---

* 'Pflüger's Archiv,' vol. 17, p. 238, 1878.

† *Ibid.*, vol. 59, p. 206, 1894.

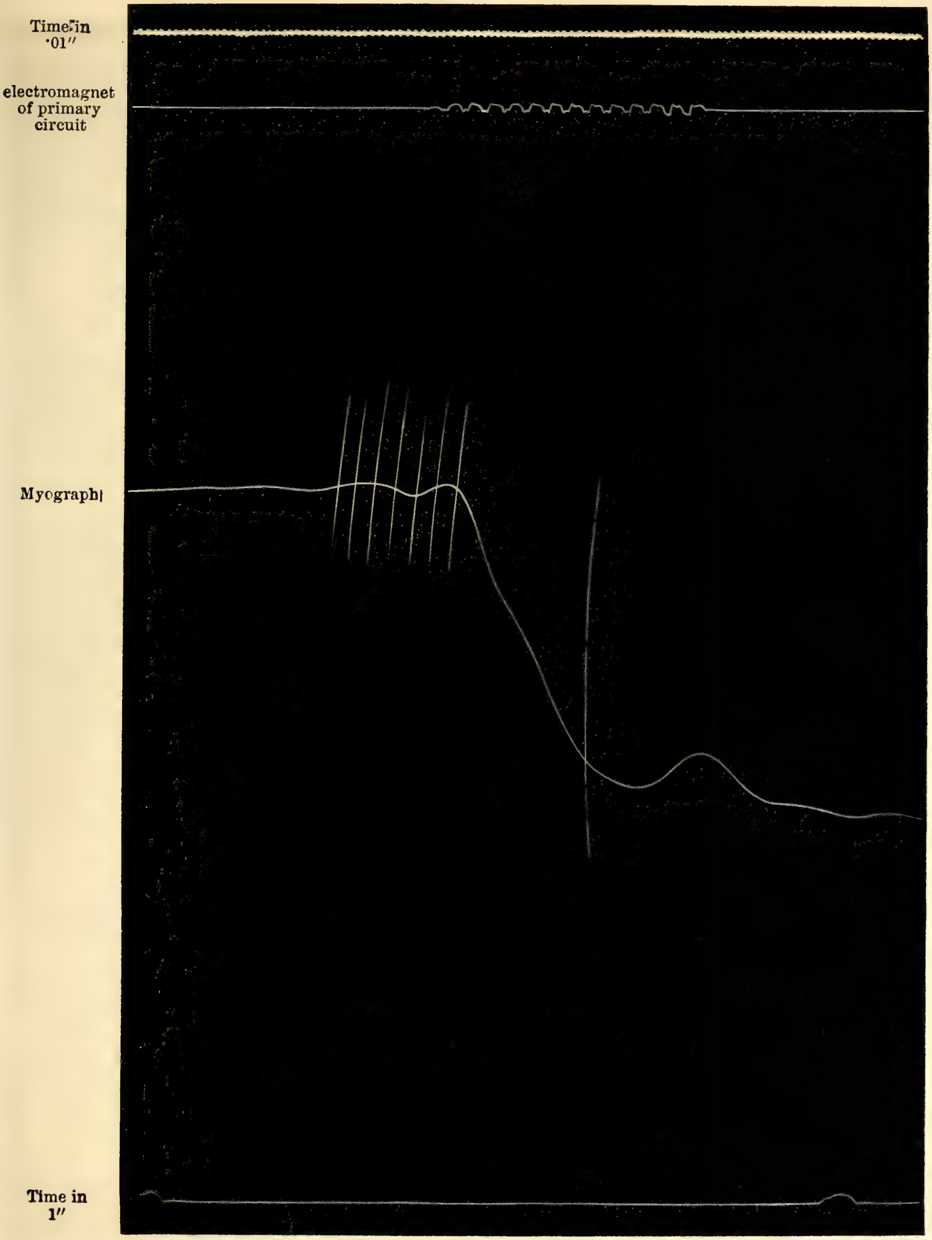
‡ W. Stirling, 'Arbeiten a. d. Physiol. Anstalt z. Leipzig,' 1874, p. 239.

Fig. 1a.



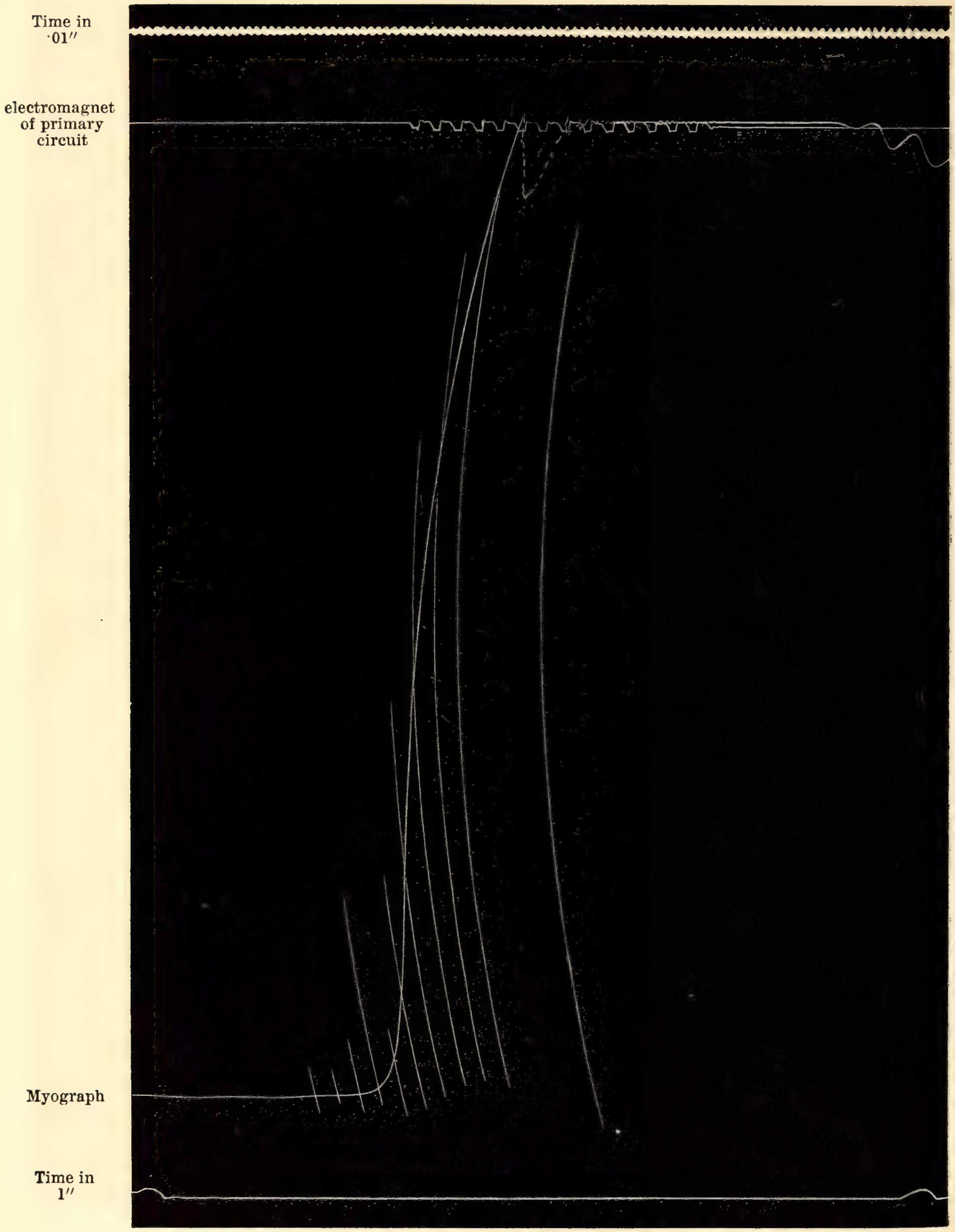
Figs. 1a and 1b.—The "flexion reflex" observed as reflex contraction (excitation) of the flexor muscle of the knee (fig. 1a) and as reflex relaxation (inhibition) of the extensor muscle of the knee (fig. 1b). The afferent nerve stimulated is a twig of the internal saphenous below the knee. The stimulation is by a series of break induction currents, the number and frequency of which is shown by the electromagnetic record of the breaks and makes of the constant current feeding the primary spiral of the inductorium through a rotating key. The distance of the secondary

FIG. 1B.



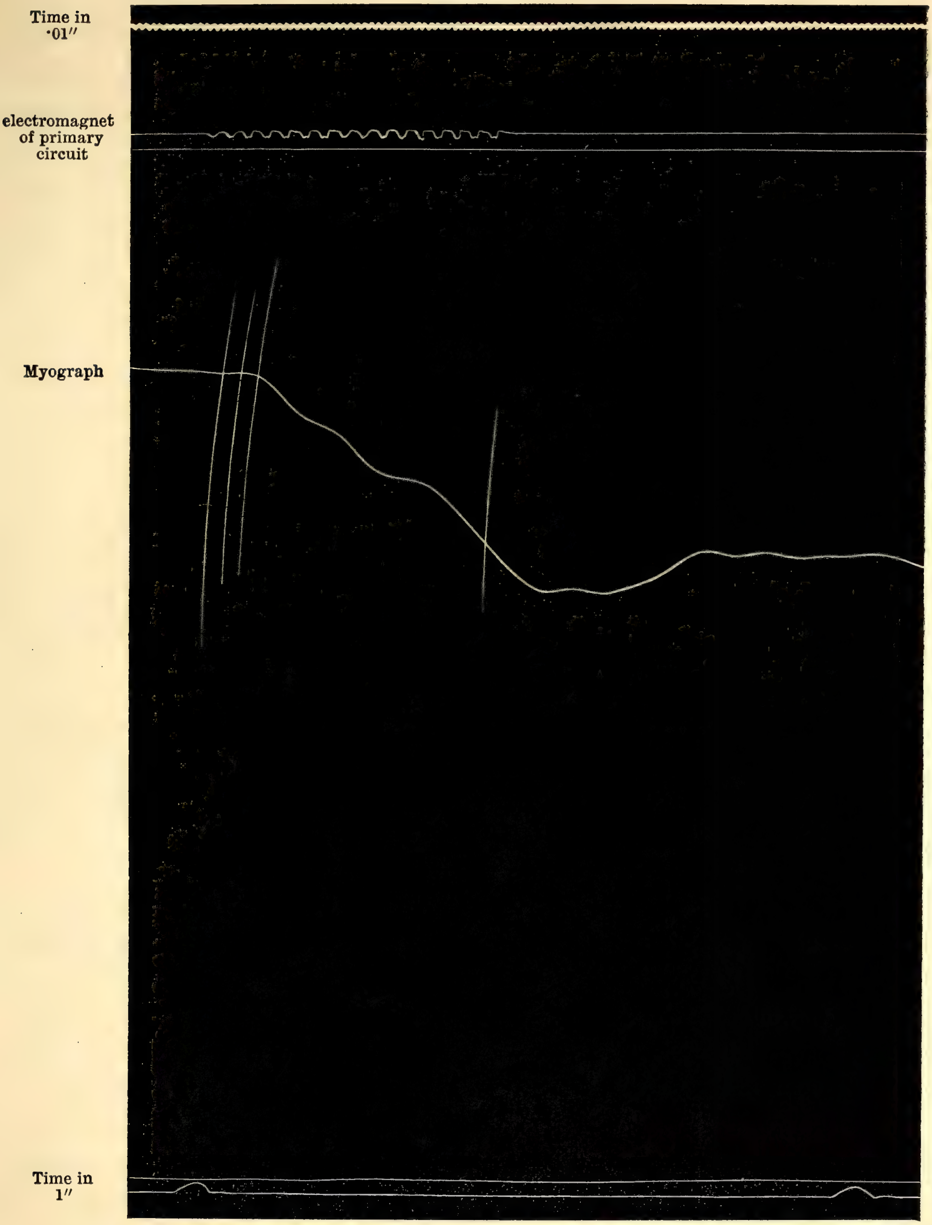
coil from the primary remained the same in the two observations (figs. 1A and 1B). The observation of fig. 1B was made from the same preparation as fig. 1A and about 4 minutes later. The moment of delivery of the individual stimuli is marked by the abscissæ on the myogram : in fig. 1A six were delivered before the reflex contraction set in ; similarly in fig. 1B, six were delivered before the reflex relaxation set in. The intensity of the stimulating shocks was feeble, hence the relatively long latent period. Time recorded in hundredth seconds above, in seconds below.

Fig. 2A.



Figs. 2A and 2B.—The "flexion reflex" observed as reflex contraction (excitation) of the flexor muscle of the knee (fig. 2A) and as reflex relaxation (inhibition) of the extensor muscle of the knee (fig. 2B). Conditions the same as in fig. 1, except that secondary coil of inductorium is nearer to primary, and therefore stimulation more intense. The latency is therefore shorter than in the pair of observations yielding fig. 1. In fig. 2A the first three stimuli fall within the latent period; in fig. 2B the first two

FIG. 2B.



stimuli only. The reflex contraction excited is more vigorous and prolonged than with the weaker stimuli of fig. 1. (The myograph lever at the top of its ascent has touched the carrier of the electromagnetic signal, and its further record is retarded until it begins to descend.) Electromagnetic records of interruptions of constant current in primary circuit, and of time, as in figs. 1A and 1B.

and in the reflex exhibited as contraction in the knee flexors of the other limb, good agreement is found; the number has been often actually the same, though the observations are made alternately first one on one limb, then one on the other limb. Figs. 1A and 1B, and 2A and 2B are such pairs, and illustrate the kind of agreement.

(θ) The course of the "flexion reflex" as shown in myograms differs much from that of certain other reflexes of the limb, notably from the "extensor thrust" and from the "scratch reflex." Its duration broadly speaking follows more closely than that of those reflexes the duration of the eliciting stimulus. If the stimulus is quite brief and not intense the myogram shows but a short continuance of the development of the effect after the external stimulus itself has ceased. I have instances of this which appear not fully explicable by inertia of recording apparatus. The after-effect is longer when the stimulus is more intense. In both respects the reflex inhibition approximates to the reflex contraction. The "flexion reflex" by adjustment of the intensity of the stimulus can be graded as to its amplitude. This grading is seen not only as a grading of the amplitude of contraction of the flexors when the stimulus is applied to the limb with intact knee flexors, but as a *grading* of the amplitude of relaxation when the stimulus is applied to the limb with intact knee extensors.

These correspondences support the view that the reflex inhibition (relaxation) and the reflex excitation (contraction) are part and parcel of one and the same reflex reaction; and that although opposite in direction they are co-ordinate reciprocal factors in one united response.

II. It was previously shown that stimulation of the afferent nerve of the flexor muscles of the knee inhibits the knee jerk elicitable from the extensor muscle of that joint;* and that it inhibits the tonus of that muscle.† In the case of certain other muscular combinations it was further shown that the reaction which throws one set of muscles into active contraction can inhibit not only the tonus of the antagonistic set but also can cut short their active contraction.‡ I find this inhibition of an active reflex contraction in the antagonistic muscles is also demonstrable as a spinal reflex with the muscles at the knee joint. It can be studied by stimulating the central end of the hamstring nerve during the production of a "crossed extension reflex" elicited by appropriate stimulation of the opposite leg (figs. 3 and 4); or by stimulating under similar circumstances, namely during production of the

---

* 'Roy. Soc. Proc.,' vol. 52, 1893.

† *Ibid.*, vol. 60, 1896.

‡ 'Journ. of Physiol.,' vol. 17, 1894; 'Proc. Physiol. Soc.,' March, 1904, Address to Section I, Brit. Assoc., 1904.

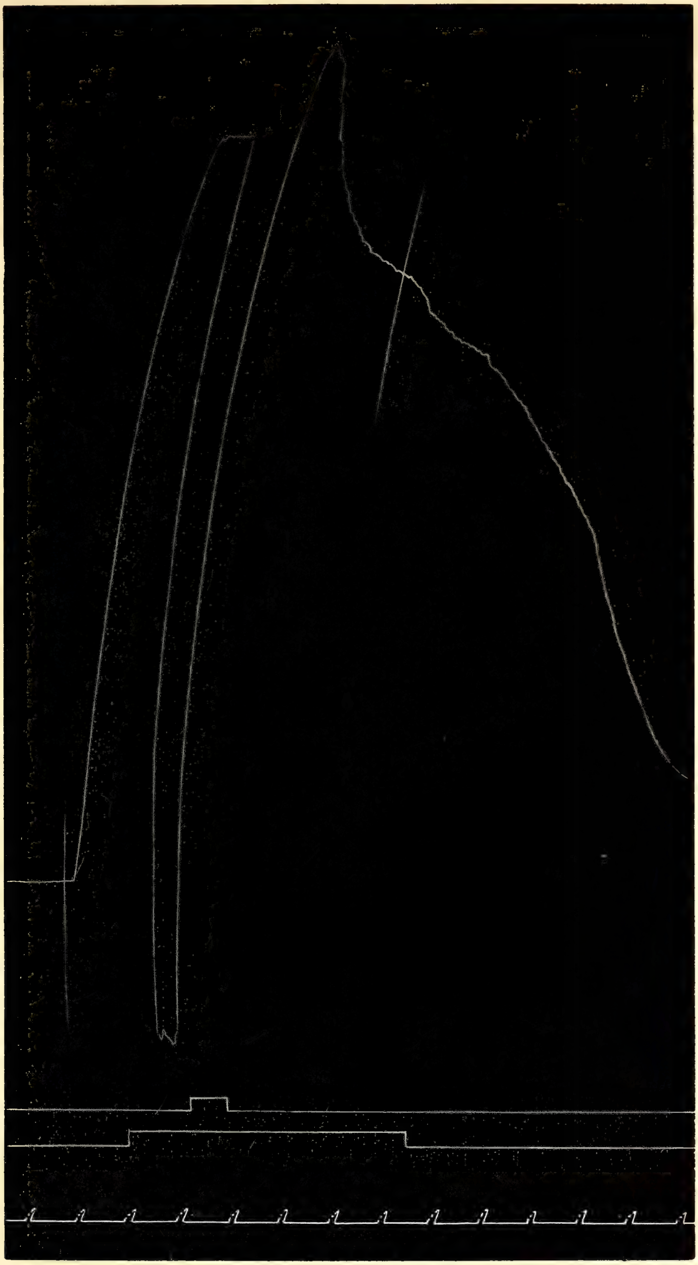


FIG 3.—Myograph record of reflex contraction of extensor of knee interrupted by a reflex inhibition (relaxation). The reflex contraction was induced by stimulation (unipolar faradisation) of the skin of the opposite foot : this stimulation was applied during the time marked by the lower signal ; its movements of commencement and ending are marked by abscissæ on the myogram. Towards the height of the reflex contraction a brief stimulation (unipolar faradisation) was applied to the skin of the foot homonymous with the knee extensor yielding the myogram : the duration of this inhibiting stimulus is marked by the upper signal. The knee extensor at outset was in some tonic contraction due to "decerebrate rigidity." The reflex inhibition relaxes this in addition to inhibiting the current reflex from the crossed foot. Time is marked below in fifths of seconds.

"crossed extension reflex," an afferent nerve or point of skin suitable for exciting the "flexion reflex" in its own limb. The same is demonstrable also in the fore limb with the muscles acting antagonistically at the elbow. The phenomenon can be also obtained at the knee in the direction reverse to that illustrated by the above example; while an isolated hamstring muscle is recording its reflex contraction by the myograph in response to appropriate stimulation of the skin of its own limb, the "crossed extension reflex" is induced. Inhibition of the active reflex contraction of the hamstring muscle occurs, and its extent can be graded as mentioned above and illustrated in the tracing figured here (fig. 5).

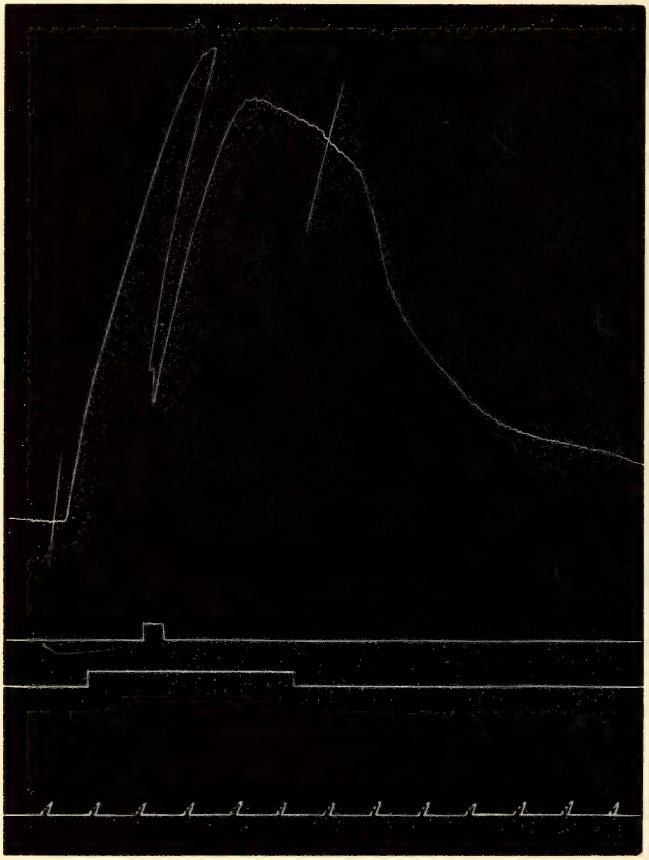


Fig. 4.—Similar to fig. 3, except that the inhibiting stimulus was weak faradisation applied to the proximal end of the severed "hamstring nerve."

The inhibition can take effect either during the application of the stimulus that excites the reflex inhibited or during the after-discharge of that reflex. In the former case the interrupted reflex contraction returns—at least it does so in many instances—if the inhibiting stimulus is discontinued before the discontinuance of the stimulus inducing the reflex contraction (figs. 3 and 4). In the latter case, namely, that of inhibition of reflex " after-discharge," the reflex contraction is often permanently cut short (fig. 5). The inhibitory effect differs markedly from the effect of mere cessation of the stimulus exciting the reflex contraction (figs. 3, 4, 5, and 6). It acts more quickly, and, if strong, it relaxes the antagonistic muscle to a greater extent than does mere cessation of the stimulus exciting the active contraction. It depresses not only the active reflex contraction, but the degree of reflex tonus of the muscle on which the active reflex contraction was superadded (fig. 3, and the right hand example in fig. 6). It relaxes the muscle even down, as was said in a previous Note, to its *post-mortem* length.

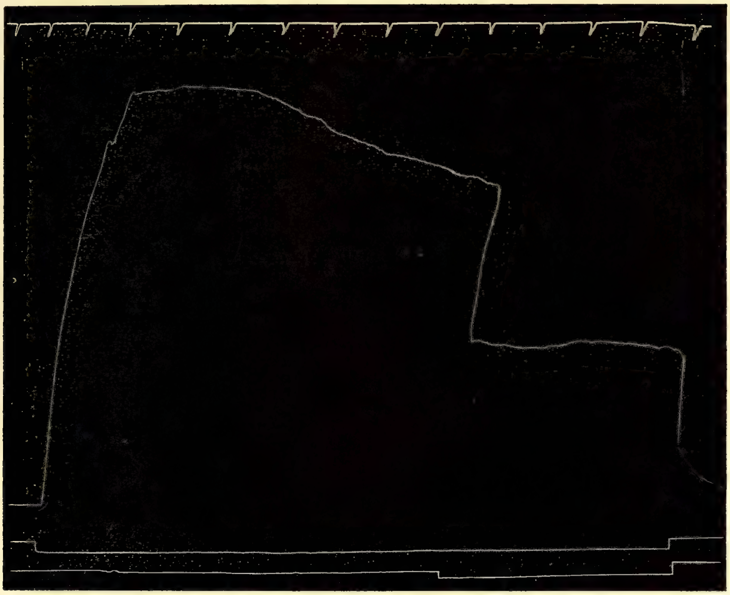


Fig. 5.—Myograph record of reflex contraction of *semimembranosus* induced by stimulation (unipolar faradisation) of the skin of the homonymous foot. The duration of this stimulus is marked by the upper signal. The lower signal marks the time of application of a stimulation (unipolar faradisation) of the skin of the contralateral foot: this stimulation caused immediate relaxation of the contracting hamstring muscle, but the relaxation did not proceed beyond a certain grade. Time is marked above in fifths of seconds.

It is interesting that this inhibition of reciprocal innervation is seen in many occasions to steady a muscle which under experimental interference with the muscles and nerves of the part is exhibiting tremor and twitchings. This effect is seen in fig. 7, where the *semitendinosus* muscle during the course of an experiment on the nerves of the hind limb of a dog, the spinal cord of

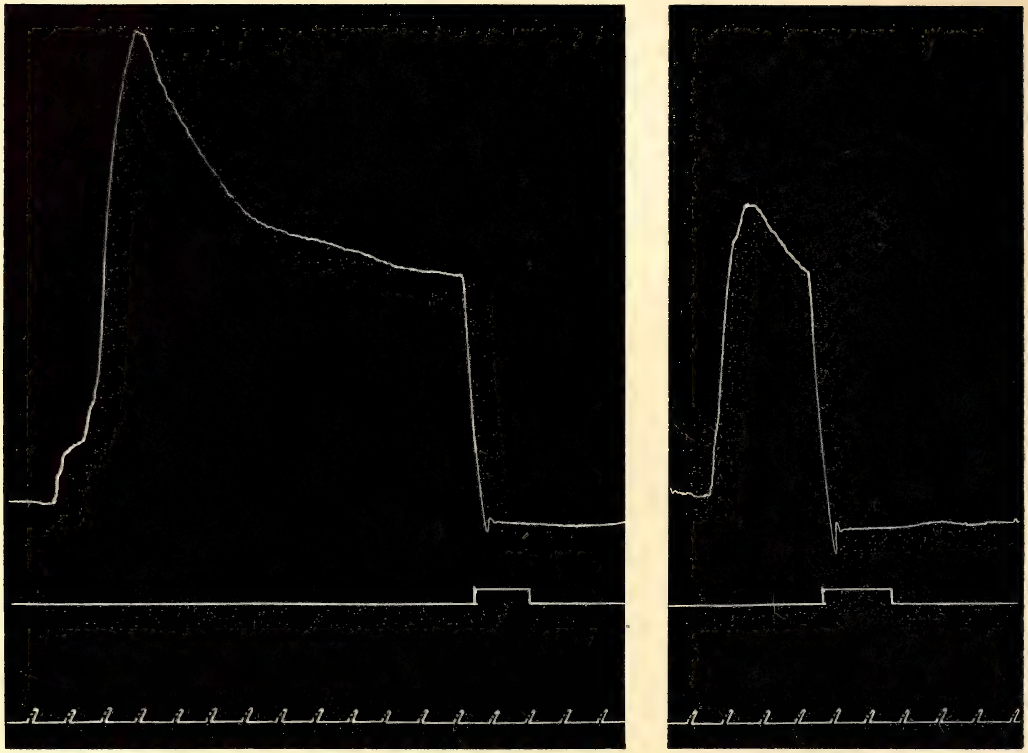


Fig. 6.—Myograph records of reflex contractions of the extensor of the knee in "decerebrate" cat. The exciting stimulus was, in the observation reproduced on the left of the figure, a brief compression—lasting less than a second—of a digit of the contralateral foot. After this stimulus had been given and discontinued, and while the after-discharge of the reflex was still in progress, the proximal end of a branch of the severed hamstring nerve was stimulated by faradisation for about a quarter of a second. The time of this inhibiting stimulus is marked by the signal. The reflex after-discharge is seen to have been at once inhibited and in this case not to have returned.

The observation reproduced on the right was from the same experiment, but later; in it the stimulation exciting the reflex contraction was faradisation of the proximal end of a twig of the internal saphenous of the contralateral leg. This stimulation lasted about two-fifths of a second or less. Its cessation was quickly succeeded by faradisation of the proximal end of a branch of the severed hamstring nerve as in the previous observation. The signal marks the time of this inhibiting stimulation. The after-discharge of the contraction reflex is cut short as before.

Time is marked below in fifths of seconds.

which had been transected at the tenth thoracic segment, early exhibited the irregular contractions shown. Considerable depth of chloroform narcosis did not appreciably reduce these contractions, though that they were of spinal origin and probably reflex is indicated by their arrest on stimulating the central end of any nerve of the opposite leg causing the "crossed extension reflex."

III. Faradisation of the cutaneous nerves of the limb generally depresses the knee-jerk, as was first shown experimentally by Sternberg.* That that influence can be exerted by these nerves has recently been laid stress on by R. du Bois Reymond. I have noted their similar effect on the *tonus.* Inhibition of the tonus of the extensor muscles has also been shown† to be obtainable from other afferent nerves besides the hamstring nerve, and notably from skin nerves of the homonymous hind limb itself. If with stimulation of one of these nerves stimulation of the hamstring nerve is combined, the stimulation of the latter is found to reinforce the inhibitory effect of the

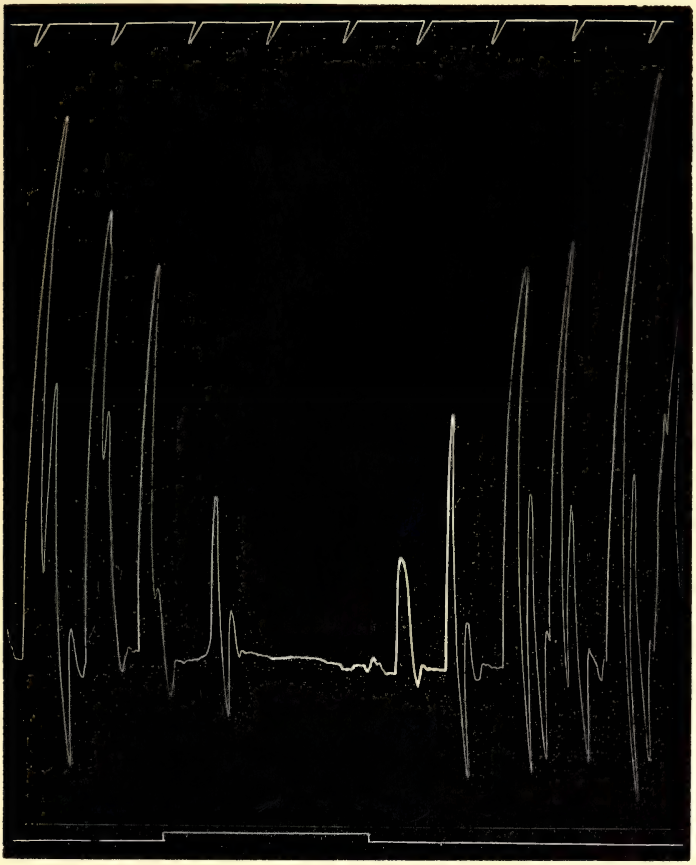


FIG. 7.—Myogram of convulsive twitching of *semitendinosus* in a "spinal" dog. The spasms are reduced and temporarily suspended by stimulation (faradisation) of the proximal end of a branch of the internal saphenous nerve of the contralateral leg. The time of application of the inhibiting stimulus is shown on the signal line below. Time is marked above in seconds.

---

* "Sehnenreflexe," Vienna, 1893; 'Roy. Soc. Proc.,' vol. 60, 1896.

† Sherrington, 'Roy. Soc. Proc.,' vol. 60, 1896.

former, and *vice versâ*. The skin nerves in question all excite reflex contraction of the hamstring muscles. I have shown* experimentally that massage and other mechanical treatment of the exposed hamstring muscles themselves discharge centripetal impulses up their afferent nerves, and that these centripetal impulses inhibit the tone of the antagonistic extensor muscle. Verworn† has demonstrated the same thing for the flexors of the ankle in respect to the *extensor digitorum pedis communis*, the antagonist to the flexor. There are experimental grounds for thinking that the contractive activity of the hamstring muscles themselves discharges centripetal impulses up their afferent nerves, and that these impulses will reinforce the inhibitory action of the skin-reflex upon the extensor muscles which is antagonistic to the flexors set in action. The reflex inhibitory action of the skin nerves in question will in that way be supplemented by the afferent nerves of the muscles that the stimuli to the skin excite reflexly to contraction, and all this will occur together as a spinal reflex. The reflex arcs arising in the skin, and the reflex arcs arising in the muscles which those cutaneous arcs excite, are, therefore, synergic; they are what I have termed "allied arcs,"‡ and their actions mutually reinforce.

IV. The knee-jerk, though there are objections to considering it a true reflex, is, nevertheless, dependent on the integrity of a reflex spinal arc. It was shown that this reflex arc in the case of the *vastus medialis* and *crureus*—the chief muscles involved in the jerk—arises in and returns to end in that very muscle itself,§ and that all other reflex arcs are inessential to the phenomenon. Hence, regarding the knee-jerk as an index of the reflex spinal tonus of the muscle, it was argued|| that the reflex arc which maintains the tonus of the extensor muscle arises in that very muscle itself. But not all authorities allow that the knee-jerk is of itself a true index of the reflex tonus. Now in "decerebrate rigidity" the tonus of the extensor muscle is so great that it is of itself, without appeal to the phenomenon of the knee-jerk, easily observable by mere inspection. Using the *vastus medialis* and *crureus* under decerebrate rigidity, I find that the tonus of those muscles—which for the present purpose can, without artificiality, be considered one muscle—persists practically unaltered after severance of all the afferent nerves of both hind limbs, excepting only the nerve of that muscle itself, either in one limb or in both. On then cocainising or severing the afferent fibres from that muscle itself, but leaving intact the efferent

---

* 'Roy. Soc. Proc.,' vol. 52, p. 556, 1893.

† 'Arch. f. Physiologie,' Supplem. Band, 1900, p. 117.

‡ 'Brit. Assoc. Reports,' Cambridge, 1904, Address to Section I.

§ 'Roy. Soc. Proc.,' vol. 52, p. 557, 1893.

|| *Ibid.*

to it, the tonus is at once and definitively abolished. To sever the afferent nerve-fibres of the muscle without interfering with the efferent, it is, of course, only necessary to sever the dorsal spinal roots in the vertebral canal—these roots in the dog and cat are the 4th, 5th, and 6th lumbar, and in the monkey the 3rd, 4th, and 5th lumbar. The reflex tonus of this muscle is, therefore, shown in this way to be unquestionably due to the nerve arcs arising in and returning to itself only.

If one of the longer branches of the hamstring nerve be carefully isolated and its central end stimulated, the excitation provokes reflex contraction in the other hamstring muscles. It never, in my experience, normally provokes contraction in the antagonistic extensor of the knee, although it does in flexors of the hip, which are anatomically closely connected with certain extensors of the knee. If, conversely, the central end of the nerve of a pure extensor of the knee, *e.g.*, *vastus medialis* and *crureus*, be similarly excited, it does not, in my experience, provoke any primary reflex contraction in the antagonistic hamstring muscles. It can, in my experience, provoke reflex contraction of the extensor muscle of the knee, but the risk of escape of current to the other branches of the nerve, when applying electrical stimuli to these branches of the anterior crural, is considerable, so that I have resorted to mechanical stimulation, and the reflex effect produced by that mode of stimulation of these muscular branches of the cruralis is slight. That mechanical stimulation of the extensor of the knee does induce reflex contraction of the extensor itself is, however, plainly demonstrable under "decerebrate rigidity." All the nerves of the limb being severed, except those to the *vasti* and *crureus*, the animal is inverted and the knee then gently but fully extended by raising the foot, the thigh being held vertical. The foot is then released, the anticrus falls, and in doing so is seen to be suddenly checked by exciting a contraction of the extensor of the knee. This contraction is different from a knee-jerk, for it only slowly passes off.

It seems, therefore, that in the case of these two groups of antagonistic muscles, the reflex contraction elicitable through the afferent nerve of the hamstrings takes place in the hamstring muscles themselves, and does not involve the opposed extensor muscle of the knee joint; and that the reflex contraction elicitable through the afferent nerve of the extensor muscle takes place in the extensor muscle itself, and does not involve the hamstrings, the opposed flexors of that joint.

V. The intimate nature of the process which reveals itself as inhibition is admittedly obscure in these reflexes as elsewhere. The present note is no place in which to review theories of inhibition advanced by Hering, v. Cyon, Gaskell, Lauder Brunton, Meltzer, Verworn, Fano, Macdougall, H. E. Hering, and others, who have written with authority on the subject. One point, however, impresses the observer in studying the phenomenon as seen in these reflexes. The process, although converse in effect to that which "excites" through the motor neurone a "contraction-reflex," yet resembles that in time-relations of onset and other ways sufficiently to suggest that the induction of the change of state in the "centre," if active in one case, is active—not passive—in the other. The throwing out of action seems, at least, as quick as the bringing into action.* H. E. Hering and myself† thought, with cortical reactions, the relaxation might commence a little sooner than the contraction of the muscles.

In the spinal reflexes dealt with here the inhibition in many cases appears not equivalent to merely arresting the play of an excited afferent channel upon the motor centre. Were that all, the phenomenon should resemble the effect of suddenly stopping the stimulation of the afferent nerve causing the reflex. What happens is often not like that; the arrest is more rapid. It is usual in spinal reflexes for the reflex contraction to endure for a time after cessation of the excitation of the receptive surface or afferent channel. This continuance of action of the motor neurone, this "after-discharge," is often marked in spinal reflexes—I have seen it continue for 20 seconds in the "flexion reflex." It seems natural to attribute it not to an after-action of the peripheral afferent nerve, but to a continuance of action by mechanisms excited by that on its central side. These mechanisms or mechanism may be internuncial between afferent channel and motor neurone, or may be the motor neurones themselves, or the synapse between any of these links. This "after-discharge," whatever its seat, can be at once arrested by the inhibition. It sometimes returns again if the arresting stimulus be brief. But the fact of its arrest shows that the inhibition acts in these cases differently from a mere discontinuance of the excitation of the afferent channel. Its effect differs from mere cessation of the exciting stimulus. The seat of this inhibition seems, therefore, to lie at the central end of the afferent neurone.

Observations on the scratch reflex‡ indicate that in that reflex the motor neurones of the flexor muscles of the hind limb can be excited to the clonic discharges characteristic of the reflex at a time when the "flexion reflex" is inhibited from employing them. When the scratch reflex is in progress it is more difficult to excite a "flexion reflex," and *vice versâ*. One reflex seems to be precluded from acting on a motor neurone at a time when another and *different* reflex is employing it. This is one of the data for what I have termed the "*principle of the final common path.*"* The preclusion of the motor neurone from the influence of *one* reflex does not preclude it from the action of other different reflexes, but still leaves it open to respond to the action of those other reflexes—that, in fact, appears to be one of the services of this inhibition to the organism. This seems to indicate that the motor neurone itself is not, in some cases at least, the seat of the inhibition, for if so, it would be inhibited for all reflexes; unless the motor neurone is functionally divisible, and one part of it, *e.g.*, one set of dendrites, can be inhibited at a time when another are not. The seat of the inhibition appears, therefore, with some likelihood, to lie neither in the afferent neurone proper nor in the efferent neurone proper, but in an internuncial mechanism—synapse or neurone—between them. I say "neurone proper" because a synapse would include the terminal of one neurone and the proximal part of the next.

---

* *V. supra*, § 1.

† 'Pflüger's Archiv,' vol. 68, p. 221, 1897.

‡ Sherrington, 'Brit. Assoc. Reports,' 1904, *loc. cit.*

The "after-discharge" of a "centre," with its concomitant persistence of contraction of muscles, might well be disadvantageous to the organism. That it is rapidly arrested by the inhibitory side of a succeeding reflex, is an adaptation which facilitates the successive interchange of reflexes.

Having in view this *active*, *i.e.*, non-passive, character of the initiation of change in central state which the inhibition implies, and also our power to excite opposed muscles synchronously in certain willed movements, I have, in the instances of various afferent nerves and points of skin that regularly evoke inhibition of some particular test-muscle, tried by varying the kind of stimulation, grading the intensity of the stimuli, cooling the nerve, etc., to change their effect from reflex inhibition to reflex contraction. Thus, with the knee extensor as test-muscle, I have experimented for this purpose on the skin of the leg below the knee, and on the hamstring nerve and its branches, the nerve from the inner and outer heads of gastrocnemius, the dorsal digital nerves, the anterior tibial nerve above the ankle, the internal saphenous and its branches below the knee, the external saphenous, and the plantar digitals. In all cases save in one I have been unsuccessful; the result has always remained inhibition, save in the single instance of the planta.† From that surface I found that certain kinds of innocuous mechanical stimuli—but not other stimuli—elicit regularly a reflex contraction of the extensor instead of reflex relaxation.

---

* Sherrington, 'Brit. Assoc. Reports,' 1904, *loc. cit.*

† Sherrington, 'Roy. Soc. Proc.,' vol. 66, p. 66, 1899; and 'Journ. of Physiol.,' vol. 30 p. 39, 1903.

This exception apart, the striking invariability of the reflex reactions from the various skin-points and nerve-twigs, together with the correspondences mentioned in Section I, allows an inference that the individual afferent nerve-fibres from the receptive-field of the reflex each divide in the spinal cord into end-branches (*e.g.*, collaterals), one set of which, when the nerve-

Fig. 8.

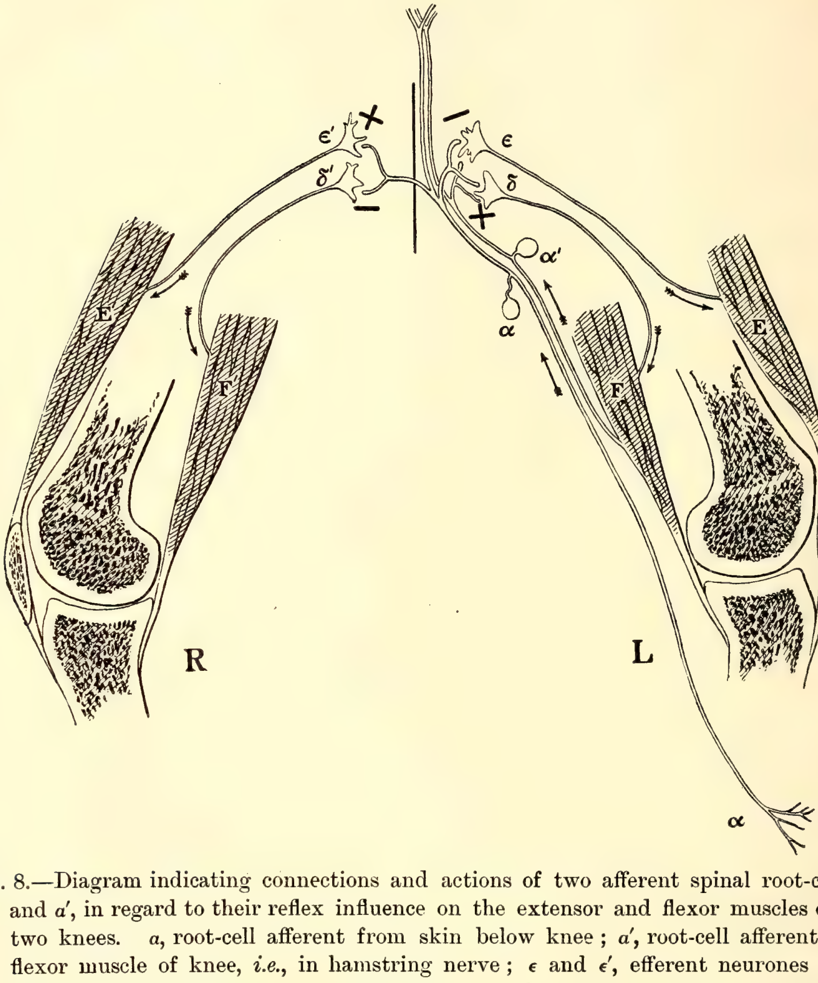


Fig. 8.—Diagram indicating connections and actions of two afferent spinal root-cells, *a* and *a*′, in regard to their reflex influence on the extensor and flexor muscles of the two knees. *a*, root-cell afferent from skin below knee ; *a*′, root-cell afferent from flexor muscle of knee, *i.e.*, in hamstring nerve ; ϵ and ϵ', efferent neurones to the extensor muscles of the knee, left and right ; δ and δ', efferent neurones to the flexor muscles ; E and E′, extensor muscles ; F and F′, flexor muscles. The "schalt-zellen" (v. Monakow) probable between the afferent and efferent root-cells are for simplicity omitted. The sign + indicates that at the synapse which it marks the afferent fibre *a* (and *a*′) excites the motor neurone to discharging activity, whereas the sign — indicates that at the synapse which it marks the afferent fibre *a* (and *a*′) inhibits the discharging activity of the motor neurones. The effect of strychnine and of tetanus toxin is to convert the *minus* sign into *plus* sign.

fibre is active, always produces excitation in certain efferent neurones, while another set of them when the nerve-fibre is active always normally produces inhibition of certain other efferent neurones, namely, those of the antagonistic muscles. The single afferent nerve-fibre would therefore be in regard to one set of its central terminal branches *specifically excitor,* and in regard to another set of its central endings *specifically inhibitory.* It will in this respect be duplex centrally. Such an inference agrees with an hypothesis which I have put forward before* regarding the mode of central termination of fibres producing this reciprocal effect. It suggests analogy between the structural arrangement for reflex reciprocal innervation and that of Astacus claw, if it be supposed that the individual nerve-fibres of the cray-fish claw preparation dichotomise, one division of the nerve-fibre passing to the opening muscle, the other division passing to the closing muscle; so that one division of the fibre exerts the excitor action, the other the well-known inhibitory, studied by Richet,† Biedermann,‡ Piotrowski,§ and others.

The constancy of the reflex inhibition remarked above is a normal constancy, but it can be upset by abnormal change of the central condition of the cord. I find that exhibition of strychnine almost at once converts the inhibition into excitation, as also, more gradually, but just as potently, does *tetanus-toxin.* This conversion sets in before and under smaller doses of strychnine or toxin than are required to produce the convulsive seizures characteristic of strychnine poisoning or general tetanus.

This transformation of effect by strychnine holds good not only for the nerves above-mentioned but for skin stimuli, and also for those skin points remote from the hind limb itself, which nevertheless, as mentioned previously, provoke reflex inhibition of the test muscle. For instance in the case of the knee-extensor as test-muscle, the skin of the fore-paws.

The conversion of inhibitory effect into excitation effect by strychnine is more easily obtained in the case of some nerves than of others. In the instances of the nerves above-mentioned the conversion is least facile, *i.e.*, requires larger doses or longer time for development, in the case of the hamstring nerve than in the others. The inhibitory effect belonging to that nerve is readily lessened by the strychnine, but its actual replacement by excitation-effect, *e.g.*, contraction of knee-extensor, not only requires larger doses of strychnine but is even then phasic rather than continuous. When this nerve is tested by stimulation at regular short intervals during one of these phasic periods it can be seen that, starting from the phase in which it still evokes inhibition, little or perhaps not at all obviously less well than normally, its inhibitory effect then becomes progressively less, until it is replaced by excitation-effect (contraction) at first mild, later violent. This periodic phase will repeat itself many times.

---

* Sherrington, 'Text-book of Physiology,' edited by Schäfer, vol. 2, 1900; *cf.* also H. E. Hering, 'Ergebnisse der Physiologie,' I, 1902.

† 'Physiol. des Muscles et des Nerfs,' p. 274, 1882.

‡ 'Sitzungsb. d. Wiener Akad.,' vol. 95, 3, p. 8, 1887.

§ 'Journ. of Physiol.,' vol. 14, 1893.

The conversion of inhibition effect as tested on the knee-extensor might be attributable to the afferent nerves stimulated containing two kinds of afferent fibres admixed, one kind causing reflex contraction of the muscle, the other kind reflex inhibition. Strychnine might, by augmenting the action of the former or by depressing the action of the latter, change the effect of stimulation of the mixed nerve. But the latter fibres would be expected to be associated in their action with—or, as urged above, to be even the selfsame fibres which evoke—contraction of the flexor muscles. Now there is at the stage of strychninisation at which the change of inhibitory into excitatory effect occurs no trace of any paralysis or even depression of the flexor contractions. As is known, and was illustrated by tracings in a former paper,* the protagonist and the antagonist muscles are together thrown into synchronous contraction as an effect of strychnine. This and other considerations appear to me to weigh against explaining the conversion of inhibition-effect into excitation-effect by the hypothesis of reflex by antagonistic sets of fibres, oppositely poisoned centrally, commingled in these afferent nerves. It is certain, however, that as shown,† the afferent nerves from the *planta* do contain, besides many fibres which evoke inhibition of the knee-extensor, some which can evoke under mechanical stimulation of the *planta* reflex contraction of the extensors of the knee. In the case of the other afferent nerves mentioned above, *e.g.*, the dorsal digitals, the hamstring nerve, the internal saphenous below the knee, the external saphenous, no evidence has ever been forthcoming that they contain any afferent fibres that can normally provoke any other effect on the knee-extensor than inhibition. Moreover, when a hamstring muscle is taken as the test-muscle, a similar conversion of inhibition into excitation (contraction) by strychnine is seen under the crossed extension reflex. This reflex, elicitable through the skin or various afferent nerves of the contralateral hind limb, normally excites the knee-extensor to contraction and inhibits the hamstrings, the knee-flexors. Under strychnine its reflex inhibition of the hamstring muscle is converted into reflex excitation (contraction) of that muscle. In view of my observations as they stand at present, I incline to the inference that the action of the alkaloid is to convert in the spinal cord in these instances the process of inhibition—whatever that may essentially be—into the process of excitation—whatever that may essentially be. The nexus was pre-existent, but the effect across it was signalised by a different sign—*minus* prior to the strychnine or tetanus toxin, instead of *plus*, as afterward.

---

* Sherrington, 'Journ. of Physiol.,' vol. 13, 1892.

† 'Roy. Soc. Proc.,' vol. 66, p. 66, 1899; and 'Journ. of Physiol.,' vol. 30, p. 39, 1903

The observed difference between the facility with which strychnine converts the inhibition by the hamstring nerve into excitation, and that with which it converts the inhibition of the other limb-nerves mentioned, does not seem referable to a different action on *muscular* afferents and *cutaneous* afferents respectively. Stimulation of the central end of the purely muscular nerve to the *vasti* and *crureus* evokes normally inhibition of the hamstrings of the opposite limb, but under strychnine it evokes their contraction. In that case, therefore, the strychnine converts with facility the inhibition by a muscular afferent into excitation, just as with the *skin* nerves mentioned.

If strychnine can convert a central inhibition into an excitation, and if the various normal reflex spinal inhibitions show differences, one from another, in the ease with which their mechanisms undergo conversion into excitatory mechanisms by strychnine, the synchronous excitation of antagonistic muscles in certain willed actions becomes, perhaps, less difficult to understand. Vaso-depressor reflexes under chloral (v. Cyon), chloroform (Bayliss), etc., change into vaso-constrictor under curare, morphia, etc. But the reversal does not appear to occur with equal facility in all afferent nerves alike. It is stated to be impossible to obtain any vascular reflex but a depressant one from the "depressor" nerve. This nerve, arising in the heart (v. Cyon)* and aorta (G. Köster and A. Tschermak),† may in a sense be considered the afferent nerve of the muscle antagonistic to the ring-musculature of the arteries, namely, the muscle whose tonus it reflexly depresses. It is in that way comparable with the afferent nerve of the hamstring muscles in relation to the extensors of the knee. The depressor action of the hamstring nerve on the knee-extensor seems, as just said, in my experience, particularly resistant to conversion from inhibition into excitation by strychnine.

The conversion of spinal inhibition into excitation by strychnine explains the simultaneous contraction of large inharmonious groups of muscles in strychnine convulsions. It also explains the occurrence, under a given stimulus, of reflex contraction of muscles that previously do not seem, under superficial examination, to be reached by the reaction. These muscles are really included in the reflex effect normally, but the effect on them then being *inhibition*, it passes unnoticed, unless special means are adopted for seeing it.

---

* 'Ludwig's Arbeiten,' Leipzig, 1866, p. 128.

† 'Pflüger's Archiv,' vol. 93, p. 24, 1902.

Thus, in the ordinary "flexion reflex," initiated, say, from the right foot, the flexion of the homonymous knee is easily seen to be due to contraction of its flexor muscles, also the concomitant extension of the crossed knee is easily seen to be due to contraction of its extensor muscles. But it requires special preparations to detect that with the contraction of the right knee-flexors there goes reflex inhibition of the right extensor, and that with the contraction of the left knee-extensor there goes reflex inhibition of the left knee-flexors. This being so, when under strychnine the reflex is suddenly changed in character, both flexors and extensors being in both legs thrown into contraction together, it appears to an observer, unaware of the previous inhibitions, that, under the strychnine, the reflex action reached muscles which it did not reach before, *e.g.*, right knee-extensor and left knee-flexor. Hence rises the hypothesis that the alkaloid breaks down a supposed spinal "resistance," previously intervening between the afferent nerves and various motor spinal cells ordinarily inaccessible to them. Strychnine does lower the threshold stimulus for spinal reflexes at one stage of its action; but the central fact of strychnine effect appears to me that it destroys spinal taxis for the skeletal musculature by upsetting the fundamental co-ordination of reciprocal innervation. It upsets reciprocal innervation because it transforms inhibition into excitation.

The vast *rôle* of inhibition in cerebral processes, as evidenced by mental reactions, and the slightness of mental symptoms in acute strychnine poisoning, indicates a difference between inhibition as it occurs in the bulbo-spinal arcs and in the arcs of purely sensual and perceptual level, a difference presumably of chemical nature.

Addendum, May 15, 1905.

At the date of the foregoing I had had opportunity for but a few experiments with tetanus-toxin, and those with one preparation only of the toxin. The results then obtained showed, as mentioned, that the toxin, as does strychnine, converts inhibition effect into excitation effect. I have since been able to make further observations, and with two other preparations of the toxin besides my own. For a solid preparation, I have to thank the kindness of Professor Roux, Director of the Institut Pasteur; for a fluid, Dr. Stenhouse Williams. The results yielded by each of these have been confirmatory of the mentions made in the Note.

The action of the toxin in respect to inhibition resembles that of strychnine closely in several ways. Thus, in the stages of the disease in which the tetanus is still "local" and manifested in one limb, namely, that (*e.g.*, the hind limb) which received the toxin injection, the toxin early converts into excitation the reflex inhibition of the extensor muscles, normally obtainable from the internal saphenous nerve, but that obtainable from the peroneal and popliteal nerves, and from the hamstring nerve, remains unreversed, though the strength of the inhibitory effect of these nerves may be very distinctly less than normal. Later, as the condition progresses, the inhibitory effect normally belonging to the peroneal and popliteal nerves becomes actually reversed into excitation. Finally, even that of the hamstring nerve itself is reversed. This is the same sequence of effect pursued by progressive increase of dosage of strychnine.

One difference that seems apparent between the action of the tetanus-toxin and the strychnine in these observations is that in the relatively slow progress of the tetanus it is easy to note a stage in which the conversion of the inhibition effect into excitation effect has occurred, while there is yet none of that obvious lowering of the threshold of reflex reaction which early marks the course of strychnine poisoning, and has been drawn attention to by many observers.

In experiments on the hind limb, I have usually introduced the toxin into the sciatic trunk well below the hamstring branch, more rarely into the hamstring nerve as well, or alone. I have found the inhibitory effect of the internal saphenous nerve (stimulated in its course below the knee) converted to excitation in 48 hours from the time of inoculation. In the gradual progress of the condition, I have several times found the hamstring nerve produce slight inhibition of the extensor if the initial posture taken at the knee be extension, and yet produce distinct excitation of the extensor if the initial posture taken at the knee be flexion. This is in accord with evidence insisted on in the sixth Note.*

It is noteworthy that the nerve, namely, the internal saphenous, which, in the lower limb, thus early shows the typical effect, *i.e.*, conversion of inhibition to excitation, lies remote from and without peripheral connection with the nerve trunk which has been the seat of inoculation. In the same way, before the condition has spread so as to cause obvious symptoms in the opposite as well as in the inoculated limb, the reflex effect of stimulation of the central end of the hamstring nerve of the inoculated limb can be seen to be at the opposite knee slight inhibition of the extensors—that is, in my experience, the normal effect, *i.e.*, obtains in the unpoisoned state. But the reflex crossed effect from the corresponding nerve of the non-inoculated limb can be seen to be not inhibition, as it normally should be, but excitation. In this case, again, inhibition effect has been changed to excitation effect, and this time in a crossed reflex, and the change is observed not on stimulating the nerve trunk actually inoculated, but on stimulating the corresponding nerve of the opposite side. This result is in accord with expectation in view of the signs of the condition present.

---

* 'Roy. Soc. Proc.,' vol. 66, 1900.

Conversion of inhibition into excitation by tetanus-toxin is demonstrable, as is that by strychnine, with the reflexes of the fore limb as well as with those of the hind limb, and in the "decerebrate" animal as well as in the merely "spinal."

The fact that the very motor neurones, which are so regularly inhibited in the type-reflexes that have here been under examination, do under certain cortical stimulations exhibit excitation instead of inhibition, was a consideration which induced my search for the possibility in them of converting inhibition into excitation. This conversion of reflex inhibitory effect into excitatory having been obtained, it has been natural to enquire what difference, if any, is thus made to the reactions of the cortex cerebri. The material for doing so required a little time in preparation, but I am now able to report that the agents found to convert the spinal reactions do exert marked influence on those of the cerebral cortex. They transform the physiological topography of the motor cortex.

It is, in my experience, quite exceptional to obtain primary extension of the opposite knee as a motor reaction from the cerebral cortex of the cat—or even, indeed, as a secondary movement. In exploring that cortex with unipolar faradisation I have often failed to elicit the movement at all throughout series of observations. Flexion, on the other hand, is regularly obtainable. This means, in light of observations by H. Hering and myself, not that this cortex is in touch with the flexors alone, and not with the extensors, but that its usual effect on the latter is *inhibition:* the extensors are not unrepresented, but their normal representation is in the form of inhibition, not excitation, and thus, unless specially sought, escapes observation. After exhibition of strychnine extension of knee becomes elicitable regularly from the cortex, and from the very points of it that yielded flexion previously. This conversion is, in my experience, not so facile as the conversion of the spinal reflex. The dose of strychnine has to be larger, or to operate longer. With doses additively given there is, early in the experiment, a period when reflex spinal inhibition of the extensors has been converted into excitation, but the cortex of the brain still yields knee-flexion, not extension. The cortical reversal has required in my hands doses that evoke convulsive seizures from time to time, and I have seen immediately after a severe convulsion the cortex either unable to evoke any movement of the knee or produce knee-flexion, though a short while before it gave knee-extension.

Tetanus toxin likewise converts the cortical flexion into extension. The effect is in its case the more marked because, if the cortical examination be conducted in an early stage of the progressive malady ensuing on inoculation by a moderate dose, or conducted where the dose has been quite small, the tetanus is "local," and confined to the limb the site of inoculation, and then, if the tetanus be "local" in one hind limb, *e.g.*, the left, the appropriate area of the right hemisphere yields knee-extension, whereas the corresponding of the left hemisphere yields knee-flexion.

But these effects are better studied in the monkey. There, in my experience, to obtain primary extension of the crossed knee from the cortex is, as in the cat, extremely unusual. A number of experiments can be made without obtaining it at all. Even as a secondary movement it is extremely poorly represented in the cortex. For twenty instances of flexion at knee, it is, in my experience, often difficult to find one of extension at that joint. But, after tetanus toxin or strychnine, the whole "leg-area" of the cortex from all points of its surface will yield nothing but leg-extension, in which extension of knee is prominent, as an evident part of a primary combined movement. This is especially striking when the tetanus is still merely "local," and confined to one hind limb, *e.g.*, left. The "leg-area" of the right cortex then yields knee-extension everywhere; the "leg-area" of the left cortex yields the normal flexion results. The "leg-area" of the right cortex provokes moreover from many of its points extension of right knee and ankle, as well as of left, though less strongly. The "leg-area" of left hemisphere does this little, if at all. Under moderate faradisation, the "leg-area" in the monkey, in my experience, moves the homonymous hind limb, in addition to the crossed, very slightly, and rarely normally, much less so than in the cat, though in both the movement is the same, namely, "extension." So localised may be the toxic influence in its early stage that reversal of its cortical effect at knee may obtain while in the same hemisphere that on hip and ankle still remain flexion as usual.

Similarly with the "arm-area." In the cat, it is in my experience quite infrequent to obtain primary extension of the crossed elbow from the cortex. Flexion is readily and regularly obtained. Strychnine changes this: the very surface that yielded flexion then provokes extension, and strongly. But the dose of strychnine has to be larger than for conversion of the spinal reflex, and the conversion shows the phases before mentioned in regard to the knee-inhibition and its conversion in the case of the hamstring nerve. In the monkey, in my experience, the effect of strychnine and of tetanus toxin when pushed to the general convulsive stage, is often contrary to the effect in that stage in so many other animals. I have, namely, seen them produce, not extension at elbow but flexion at elbow. That is, however, not invariable. In one case, in a monkey, in which the tetanus had become general in the sense that only one limb was unaffected, the affected arm was strongly extended and rigid at elbow with some retraction at shoulder. But in all my instances of " local " tetanus of the arm by introduction of the toxin into the trunk of the median the limb has been extended rigidly at elbow and retracted at shoulder. In these faradic examination of the cortex has shown that the small field of the "arm-area," to which extension at elbow is restricted, was enlarged so as to include the whole " arm-area." Extension at elbow sometimes alone, more often with retraction at shoulder, or with extension at wrist or fingers, sometimes as a leading movement, sometimes rapidly ensuent on retraction at shoulder or extension in the hand, according as higher or lower points in the area were stimulated, was prominently exhibited at all points of the entire surface of the " arm-area." That area, with this as its salient reaction, seemed particularly excitable, for its extreme limits appeared traceable further than usual, and encroaching on or overlapping more than is usual, under the feeble or moderate stimulation employed, the " leg-area " above and the " face-area " below, and to run exceptionally far forward above the pre-central sulcus, though remaining undemonstrable in the free surface of the ascending parietal convolution. From no point in all this extensive " arm-area " was, despite repeated trials, any flexion at elbow or at shoulder or hand obtained. Various intensities of faradisation were employed in attempt to evoke it, and points known to normally yield it very regularly were specially tried: but extension, not flexion, always resulted.

This condition of the " arm-area " I found in experimental tetanus sometimes exist in one hemisphere or even in both hemispheres, while the " leg-area " of each hemisphere yet yielded flexions at knee and hip and ankle, and its other normal forms of reaction. In the monkey, in my experience, tetanus produced by introduction of the toxin into the arm (*e.g.*, median trunk) affects after the inoculated limb, the fellow fore limb first, and the jaw before the hind limbs, although the knee-jerk on the homonymous side to the inoculation may be brisk.

Under decerebrate rigidity, *e.g.*, in the cat, the closing muscles of the jaw are kept in tonic action, holding the mouth somewhat firmly shut.* By stimulation of any point of a large " skin-area " appropriate for the reflex, reflex opening of the mouth, including depression of the lower jaw is easily and regularly elicited. Faradisation of an afferent twig of the trigeminus produces the same effect; or as was shown by Woodworth and myself,* stimulation of even distant afferent nerves, *e.g.*, plantar or saphenous. Here the action of the powerful closing muscles is reflexly inhibited while the weaker opening muscles are reflexly excited—it is, in fact, a case of Astacus claw, except that the inhibition is central, not peripheral. This reflex "opening" is in the decerebrate animal converted into reflex closure by tetanus toxin and by strychnine, the inhibition of the predominantly powerful closing muscles being converted into excitation of them.

---

* Sherrington, 'Phil. Trans.,' B., vol. 190, 1898.

Similarly, when the "face-area" of the monkey's cortex is tested by faradisation after exhibition of strychnine, the points of surface that, prior to the drug, yielded regularly the free opening of the jaw, yield strong closure of the jaw instead. Now closure of the jaw, is in my experience, quite an infrequent movement to obtain from the cortex of the cat or monkey,† one frequently fails to elicit it by moderate stimulation from any point whatsoever. When it does present itself, it tends to be unreliable even at the point at which it may have been evoked. On the other hand, *opening* of the jaw is always readily and regularly elicitable from a large field of the "face-area." And adjoining and overlapping this large area whence steady opening of the jaw is obtained, is an area whence, as Ferrier‡ first pointed out, "rhythmic alternating opening and closing of the jaws," as in feeding, can be evoked. Under tetanus toxin or strychnine the whole of this combined area not only ceases to yield opening of the jaws, either maintained or rhythmic, but yields closing of them instead—often with visible retraction of the tongue. For this conversion larger doses of strychnine have, in my hands, been required than for conversion of knee-flexion into extension. With tetanus toxin the conversion appears the more striking when examined early in the progress of the intoxication, because it may be found at a stage precedent altogether to the occurrence of any general convulsions, and also because it can then sometimes be found to be unilateral, that is, to be present in the "face-area" of one hemisphere§ without, or almost without, any affection of the "face-area" of the other hemisphere. The reactions of the normal field thus remain for comparison in the same individual with the reactions of the abnormal field.

The tetanus toxin in my experiments in the monkey has shown marked predilection for the closure mechanism of the jaw. After inoculation in the hind leg, even before the "local" tetanus has obviously invaded the fellow limb of the opposite side, a slightly clenched jaw and an immobile pursing of the lips has several times given warning that general tetanus had really set in, long before any trace of general convulsive seizures or any involvement of the arms was detected. Tetanus toxin has also certainly intensified the reactions of the cortical areas that give retraction of the neck and retraction of the abdominal wall.

---

* 'Journal of Physiology,' vol. 28, 1904.

† The species of monkeys used have been *M. rhesus, callothrix, and cynomolgus.* No reference is here meant to the anthropoid apes.

‡ 'Functions of the Brain,' 1876.

§ The hemisphere, the reactions of the "face-area" of which are earlier affected, is in the case of inoculation in a limb the hemisphere contralateral to the limb inoculated.

The progress of the change wrought by these agents in converting these reactions of the cortex from their usual form to the diametrically opposite seems to involve the same kind of steps as that noted above in their conversion of the inhibitory hamstring nerve effect on the knee extensor. There stages can be found in which the inhibitory effect is less than normal, yet is not replaced by excitatory. So with the cortical opening of jaw, in early tetanus a grade is discoverable when faradisation of the cortex produces a slight opening of the jaw—a mere "loosening" of the jaws so to say—distinctly less than normal and hardly effectively opening the mouth. Also with the "leg-area" of the cortex, at an early stage of the tetanus it would seem that an undue but far from exclusive preponderance of plantar extension at ankle over dorsal flexion at that joint exists, while the symptomatic knee-extension is as yet not excitable though knee-flexion is almost in abeyance. Neither under tetanus toxin or strychnine have I at present observed conversion of the abducens inhibition* into excitation.

In this brief account it might appear that the effects of tetanus toxin and of strychnine on the cortical reactions seem identical. In reality a number of differences between them appear to exist. These and other points I must reserve for a more detailed communication. But the foregoing observations appear to give an insight into at least a part of the essential nature of the condition brought about by tetanus and by strychnine poisoning. These disorders work havoc with the co-ordinating mechanisms of the central nervous system, because in regard to certain great groups of musculature they change the reciprocal inhibitions, normally assured by the central nervous mechanisms, into excitations. The sufferer is subjected to a disorder of co-ordination which, though not necessarily of itself accompanied by physical pain, must inflict on the mind, which still remains clear, a torture inexpressibly distressing. Each attempt to execute certain muscular acts of vital importance, such as the taking of food, is defeated because from the attempt results an act exactly the opposite to that intended. The endeavour to open the jaw to take food or drink induces closure of the jaw, because the normal inhibition of the stronger set of muscles—the closing muscles—is by the agent converted into excitation of them. Moreover, the various reflex arcs that cause inhibition of these muscles not only cause excitation of them instead, but are, periodically or more or less constantly, in a state of hyper-excitement, and yet attempt on the part of the sufferer to restrain, to inhibit, their reflex reaction, instead of relaxing them only heightens their excitation further, and thus exacerbates a rigidity or a convulsion already in progress.

---

* Sherrington, 'Roy. Soc. Proc.,' vol. 52, 1893.

I have not yet examined in this respect the action of the virus of rabies. It seems to me not improbable that that virus also upsets reciprocal innervation, though its field of operation, at least in man, probably lies not in the same group of mechanisms as are affected by strychnine and tetanus toxin but in an allied one, namely, the co-ordinations inter-regulating deglutition and respiration.

excitation of them. Moreover, the various reflex arcs that cause inhibition of these muscles not only cause excitation of them instead, but are, periodically or more or less constantly, in a state of hyper-excitement, and yet attempt on the part of the sufferer to restrain, to inhibit, their reflex reaction, instead of relaxing them only heightens their excitation further, and thus exacerbates a rigidity or a convulsion already in progress.

I have not yet examined in this respect the action of the virus of rabies. It seems to me not improbable that that virus also upsets reciprocal innervation, though its field of operation, at least in man, probably lies not in the same group of mechanisms as are affected by strychnine and tetanus toxin but in an allied one, namely, the co-ordinations inter-regulating deglutition and respiration.

On the Dimorphism of the English Species of Nummulites, *and the Size of the Megalosphere in relation to that of the Microspheric and Megalospheric Tests in this Genus.*

By J. J. Lister, M.A., F.R.S., Fellow of St. John's College and Demonstrator of Comparative Anatomy in the University of Cambridge.

(Received March 2,—Read March 16, 1905.)

[Plates 3—5.]

(1) *The Dimorphism of the English Species of Nummulites.*

Before the dimorphic character of many of the species belonging to the higher groups of the Foraminifera was established, some of the phenomena dependent on it had been recognised among the fossil nummulites, and attention was called to the peculiar mode of occurrence in pairs of what were then regarded as the different species of *Nummulites* and *Assilina*, in the beds in which they abound.

It was especially by the careful labours of de Hantken and de la Harpe that this phenomenon was brought to light, and the "Law of the Association of Species in Pairs" was formulated by the latter in his 'Étude des Nummulites de la Suisse' (5, p. 63), as follows:—"Les nummulites apparaissent par couples; chaque couple est formé de deux espèces du même groupe zoologique et de grandeur inégale, la grande est sans chambre centrale, la petite en a toujours une."

De la Harpe proceeds to point out that the small species is usually much more abundant than the large, often furnishing 90 per cent. or more of the whole number of specimens present in the deposit.

In gathering materials for his 'Étude,' in which he proposed to deal not with the Swiss nummulites alone, but with the group as a whole, hoping to be able to introduce order into the "Babylonian confusion" in which he found it, de la Harpe received specimens of the English nummulites from Professor Rupert Jones, and in acknowledging them, wrote (October 10, 1879):—

". . . . . . (II) *N. variolaria* from Stubbington, and (III) *N. variolaria* from White-Cliff Bay, are exactly the same as the Belgian form. They belong to the type of the species.

"It is remarkable that among your specimens there is not a single *N. Heberti*, which species is nearly always the companion of *N. variolaria.* In Belgium and France, in the upper part of the *variolaria*-beds, there is usually 1 per cent. of *N. Heberti.* In the lower beds (base of the

‘Laekenian’) these are more numerous (up to 50 per cent.). Is it that *Heberti* is totally absent, or that the number of the specimens you kindly sent me is still too few for strict comparison? This is an interesting question to solve.

“(IV) My best thanks for *N. Prestwichiana*, R. Jones . . . . . . I am now working out the Belgian Nummulites. I find in that country eight species, very distinct and easy to separate.”

Here follows the list:—

I.	*N. planulata*, Lamk.,	“without central chamber.’
	N. elegans, Sow.,	“with central chamber.”
II.	*N. laevigata*, Lamk.,	“without central chamber.”
	N. Lamarcki, d’Arch.,	“with central chamber.”
III.	*N. Heberti*, d’Arch.,	“without central chamber.”
	N. variolaria, Sow.,	“with central chamber.”
IV.	*N. d’Orbignyi*, Gal.,	“without central chamber.”
	N. Wemmelensis (or *Prestwichii*).	“with central chamber.”

De la Harpe continues: “They make four pairs, of two species each, one species being without, the second with the central chamber . . . . . .

“As far as I know, *N. Heberti* and *Orbignyi* have not yet been observed in England. I should be very much surprised if they do not exist in the same beds with *N. variolaria* and *Wemmelensis* (*Prestwichii*). Their absence would be a remarkable exception to the general law of the distribution of Nummulites.” The law is then stated nearly in the form given above [10, Supl. Note viii, 2].

Although de la Harpe had done so much to bring to light the essential facts of the case, he had suggested no explanation of this remarkable law. The question of its significance remained for him “sans solution.”

In the following year (1880) Munier-Chalmas brought before the Geological Society of France his conclusion, based on the study of four fossil species of *Nummulites* and two of *Assilina*, that the species of these genera are dimorphic, and that the phenomenon of dimorphism would be found to be general (15). The explanation thus offered of the phenomenon of the distribution of the “species” in couples, was that the members of the couple were not, in fact, of distinct species, but that they represented two forms of a single species.

So far, Munier-Chalmas’ conclusion was, as subsequent investigations have abundantly proved, entirely right. It has been shown by observations on living foraminifera (13, 14, and 16) that the form with the large central chamber (megalospheric) may arise as the offspring of the form with a small central chamber (microspheric) (not "without central chamber," as stated by de la Harpe), and there are good grounds for concluding (14, pp. 74 to 77) that the two supposed "species" represent alternating or recurring generations in the life history of a single species. This, however, is to anticipate the history of the subject.

To the entirely right conclusion above stated, Munier-Chalmas unfortunately added an extension of his view which was wrong. It so happened that among the microspheric specimens of the species which he investigated he failed to find any of so small a size as that commonly attained by the megalospheric form; and he concluded that though the two sets of individuals constituting a species were in some unexplained way of different natures, yet that both began life in the guise of the megalospheric form. At a certain stage of growth, it was supposed, the individuals of one, and the more abundant set ceased to grow, while the growth of the members of the other set proceeded. On the one hand, chambers were added in forward continuation of the series already formed, building up the large test of the microspheric form; and on the other, at the centre of the test, the large central chamber was absorbed, and chambers of smaller and smaller size were laid down, continuing the spiral series in a backward direction to the centre.

In January, 1881, the first part of de la Harpe's 'Étude' appeared, and under the head of "Association of Species" (p. 65) he criticised Munier-Chalmas' conclusions. In the same month, writing in association with de Hantken, he addressed a letter to M. Tournouer, of the Geological Society of France (6) in which the same criticisms are set forth.

He had no difficulty in disposing of the second part of Munier-Chalmas' proposition. With regard to the dimorphism of the species of nummulites, he says:—"Une idée qui, sans avoir été formulée, a cependant traversé l'esprit de plusieurs, c'est que ces deux formes sœurs, toujours associées, représenteraient peut-être les deux sexes de la même espèce. Rien dans leur mode de distribution ni dans leur fréquence relative ne s'opposerait à cette hypothèse. En l'admettant tous les faits deviendraient faciles à comprendre. D'autre part, dans les Rhizopodes, non seulement les sexes ne paraissent pas être séparés, mais leurs diverses fonctions ne semblent pas même localisées. Comment donc admettre que deux Rhizopodes bâtis sur des plans différents représentent les deux sexes d'une même espèce? La question reste donc sans solution."*

Driven now, as it would appear, by the error of the second part of Munier-Chalmas' proposition, to adopt an attitude very similar to that which, as we find by his letter to Professor Jones, he would have been "very much surprised" a year and a half before to have found correct, de la Harpe proceeded to state that the law of the association of species in pairs has exceptions. He mentions *N. gizehensis, Vicaryi, obtusa,* and says that among species with small central chambers (*i.e.*, as we now say, small megalospheres), "il est plusieurs dont les homologues n'existent pas ou n'ont pas encore été découvertes. Citons *anomala,* de la Harpe, *dubia,* de la Harpe, et *subplanulata,* Hantk." Perhaps other species, he adds elsewhere, will be found "privées d'une sœur."

---

* 'Étude,' p. 65.

It is true that in the case of *N. Vicaryi* and *obtusa* de la Harpe did not speak from his own observation. They are only cited from Medlicott and Blanford's 'Manual of the Geology of India' (published in 1879), and there is no evidence that either the collectors or describers of these species were looking out for associated forms. So that these cases go for very little.

Moreover, in the second part of the work, being an "Étude détaillée des Nummulites du groupe de la *N. gizehensis* Ehrenb.," de la Harpe points out that this species forms no exception, *N. curvispirus* (a form which he had previously regarded as identical with *N. Lucasanus*) being its habitual companion, associated with it as he elsewhere (7) says, by thousands and millions.

But before leaving de la Harpe, let us hasten to call to mind that in the fourth part of the 'Étude' (consisting of a systematic account of the group of *N. Murchisoni,* a division of the "Nummulites à filets non reticulés") and published after the untimely death (in February, 1882) of this talented palæontologist he departs from the classification proposed in the first part and deals with the species in pairs, describing in each case a megalospheric form immediately after its microspheric "homologue." There is thus some ground for thinking that in the last year of his life de la Harpe returned to the sounder view of the general prevalence of the law of association in pairs which he had himself done so much to substantiate.*

Although fully convinced of the truth of the doctrine of the dimorphism of the species not only of *Nummulites,* but of most† of the higher groups of the foraminifera, I have often felt, and especially before looking into de la

---

* Since writing this I have found the following passage in a paper read by de la Harpe before the Société Vaudoise des Sciences Naturelles on September 9, 1881 :—(8, p. 437) "Est ce" (*i.e.*, the law of association by pairs) "une règle sans exception ? Oui, nous le croyons. Hâtons-nous d'ajouter que ce n'est que tout dernièrement que nous avons acquis cette conviction." He proceeds to withdraw the case of *Nummulites gizehensis,* as in the second part of the 'Étude.' No mention is made in this communication of the other exceptions insisted on in the 'Étude.'

† I say *most* in view of the peculiar character of the pelagic foraminifera, a group on which I hope shortly to have something to say.

Harpe's position afresh, that his insistence on the exceptions to the law of distribution in pairs was a difficulty which it would be desirable, if possible, to clear up. No one knew better than de la Harpe how widely the law holds good, and yet he insisted on exceptions. If there are strata in which only one form is present, how can this be reconciled with the view that both forms recur at short periods in the life-history of the species?

The difficulty appeared to be emphasised by Professor Rupert Jones' paper (11) on "*Nummulites elegans* and other English Nummulites," in which he for the first time gave an account of the characters and distribution of the English species, and cleared up the confusion which had arisen over the identity of the type specimens of "*N. elegans*," illustrated in Sowerby's 'Mineral Conchology' (20).

The matter of dimorphism is dealt with very sparingly in this paper. It was written seven or eight years after de la Harpe had expressed his surprise at the apparent absence from the English beds of the forms named *N. Heberti* and *N. Orbignyi*, the [microspheric] homologues of *N. variolarius* and *N. wemmelensis* var. *elegans* (to use the name which Professor Jones now applied to the species which he had previously (9) named *N. planulata* var. *Prestwichiana*).

Of the latter it is stated (on page 140) that it is one of the nummulites which have a large primordial or central chamber, and the megalospheric character is shown in figs. 4 and 8 of his Plate 9.

The figure of the section of *variolarius* also shows a small megalosphere at the centre, and the description of this species ends with the words "according to Dr. de la Harpe the *N. variolarius* of the Barton Beds of Stubbington and White Cliff Bay is the same as that of Belgium and typical. He did not find *N. Heberti* (having a small central chamber) with it, though accompanying it in Belgium and France."

Of *N. laevigatus* it is said, "according to Dr. Ph. de la Harpe, the Bracklesham Nummulites comprise both *N. laevigatus* and *N. Lamarcki*, the latter having a large central chamber . . . . ."

It thus appeared to be possible that "*N. wemmelensis* var. *elegans*" and *N. variolarius*, at least as they occur in our English beds, might afford some evidence of a similar nature to that of the supposed exceptions insisted on by de la Harpe; and, at any rate, so far as published descriptions went, they were, in the English beds, forms, to use his expression, " privées d'une sœur."

I therefore spent some days last September in the Isle of Wight and on the adjoining coasts of Sussex and Hampshire in search of the species of nummulites in the Middle and Upper Eocene Beds, with the purpose of examining them from the point of view of dimorphism. I will now give the results.

Nummulites laevigatus (Brug.) (*Lamarcki* d'Arch.).*

This species abounds in some of the upper Bracklesham Beds, near Selsey. It is an admirably clear example of the law of association of species in pairs or as we now say of a dimorphic species, the difference between the two associated forms being obvious to superficial inspection in the sizes they attain (Plate 3, *a* and *b*). As the specimens lie in the beds, and still more clearly on separating them, by a sieve, from the scarcely hardened glauconitic sand in which they are contained, the full grown microspheric forms (*N. laevigatus* (Brug.) proper) are at once distinguished by their large size.

The average diameter of 12 such specimens is 15·8 mm., that of 19 approximately full-grown specimens of the megalospheric form (the *N. Lamarcki* of d'Archiac and Haime) being 4·5 mm.

The relative proportion of large to small forms in four samples (containing in all some 8000 specimens) is 1 : 8·2, 1 : 16·7, 1 : 5·8, and 1 : 10, giving an average of 1 : 10. It must, however, be borne in mind that a certain proportion of the small specimens are young microspheric individuals.

On grinding down examples of the *microspheric* form to obtain sections showing the initial chamber, the diameter of this is found to be in two cases 16 and 19 μ (Plate 3, *b''*).

The megalosphere is very large in this as compared with that found in the other English species. Among 20 specimens the mean between the long and short diameters varies from 355 to 595 μ, and the average is 443 μ, nearly $\frac{1}{2}$ mm. (Plate 3, *a''*).

After this experience at Selsey, it was striking to find the specimens of the little *N. variolarius*, which are thickly scattered through a sandy band in the Bracklesham Beds at White Cliff Bay, and of *N. Orbignyi* (*wemmelensis*) var. *elegans* from the base of the Barton Beds at Alum Bay in the Isle of Wight and at other localities, all attaining an approximately uniform size. In external appearance, therefore, these species showed no indication that they conform to the law of dimorphism.

---

* In view of the fact that each of the two forms of the species of nummulites has been separately named the question arises (*cf.* M. E. van den Broeck [2]) which of the two names so applied shall stand as that of the species. There seems no reason for departing from the ordinary rules of nomenclature in this instance. I have, therefore, in each case selected for that of the species the name first given, whether to the megalospheric or the microspheric form of it. M. van den Broeck would prefer to select for the specific name that of the megalospheric form (which he, rather oddly, regards as the more "normal" form of the species); concluding that, this form being the more abundant, it will usually turn out to be that first given. As a fact, however, among the 9 species dealt with in this paper the megalospheric form was, I believe, named before the microspheric in only one, viz., *N. variolarius.*

Examination of them by section, however, yielded the following results:—

Nummulites variolarius (Lamk.) (*N. Heberti*, d'Arch.).

Among 168 specimens examined in section 163 are megalospheric, and 5 microspheric, a proportion of 33 to 1.

In the *megalospheric* specimens (Plate 4, *a*, *a'*, *a''*) the mean between the longest and shortest diameters of the initial chamber, as it appears in the section* varies, as shown in fig. 1, from 38 to 102 μ, the average size being about 68 μ. The largest specimen of this form is 2 mm. in the longer diameter of the test, the average diameter in 13 large specimens is 1·8 mm.

In the *microspheric* form (the *N. Heberti* of d'Archiac)† the mean diameter of the initial chamber ($= m$) in the five cases is 15, 16, 16, 16, and 17 μ (Plate 4, *b b'*). Specimens of this form also attain a diameter of 2 mm., and the average in five large specimens is 1·92. There is thus a slight tendency in this species for the microspheric form to exceed the megalospheric in size. (See also the table on p. 311.)

Nummulites Orbignyi (Galeotti), (*wemmelensis*, de la Harpe and van den Broeck), var. *elegans*, Sow.‡

Operculina Orbignyi, Galeotti, 'Mém. couronné. Acad. de Belgique,' T. 13, p. 54.

---

* I use M to indicate this mean value in the megalospheric form, and m in the microspheric form.

† In the 'Bull. Soc. Géol. de France,' 1881, p. 172, de la Harpe says "Enfin pour le *N. variolaria*, Sow. : le fait est plus frappant encore ; on est presque toujours obligé de briser ces petites nummulites de Paris, Bruxelles, Gand, Stubbington et Isle de Wight pour savoir si, oui ou non, elles ont une chambre centrale, et si l'on doit les ranger parmi les *N. Heberti* ou *variolaria*."

‡ Perhaps it is due to my readers to set before them as briefly as possible some of the changes of nomenclature of which this pretty little nummulite has been the victim.

In the 'Mineral Conchology,' vol. 6 (1829), p. 76, Sowerby set about describing three species of nummulites, figuring them on Plate 538. The groups of figures in illustration of each species are numbered 1, 2 and 3, though the group numbered 3 comes in the middle and that numbered 2 below. The several figures in each group are not numbered, but by counting the figures and following the order of the numbers of the groups, a number may be given to each figure. In this way the numbers 6 to 11 may be assigned to the figures in group 2, the lowest on the Plate.

The first and third species enumerated are *N. laevigata* and *N. variolaria*, and about their identity there is no question ; but unfortunately Sowerby confused two distinct species under the name *N. elegans*, constituting his second species. The two species thus confused appear to have been, as shown by comparing the figures with the specimens in the Sowerby Collection now in the British Museum :—

N. wemmelensis var. *elegans* (called *N. Orbignyi* var. *elegans* in this paper), mounted on tablets 44,007 (1 and 2), and *N. planulata* (Lamk.), said to come from Emsworth, and mounted on tablets 44,007 (3 to 5).

Nummulina planulata, Lamk., var. *Prestwichiana,* T. R. Jones. ('Q. J. G. S.,' vol. 18, 1861, p. 93.)

Nummulites wemmelensis, de la Harpe and van den Broeck, var. *Prestwichiana,* T. R. Jones. De la Harpe's "Étude des Nummulites de la Suisse," 'Mém. Soc. Palæont. Suisse,' vol. 10, 1883, p. 169.

Nummulites wemmelensis, de la Harpe and van den Broeck, var. *elegans,* Sow. T. R. Jones. 'Q. J. G. S.,' vol. 43 (1887), p. 132.

Of the latter no specimens have, in recent years, been known to occur near Emsworth, or indeed anywhere else in England, and it appears probable, as Professor Jones has pointed out (11), that in addition to confusing two species under one specific name, Sowerby assigned a wrong locality to one of them by referring to Emsworth, a village near Chichester, a specimen from a Belgian or some other Continental locality.

In 1861 Professor Jones having specimens from Bed No. 29 of Alum Bay and from High Cliff referred to him for naming, and supposing that all the specimens named by Sowerby *N. elegans* should be referred to *N. planulata*, gave the name *N. planulata* var. *Prestwichiana* to the apparently new variety from Bed 29. (9.)

In 1879 de la Harpe was, as we have seen, reviewing the species of nummulites, and received from Professor Jones specimens from Bed 29. He considered them to represent a local variety of a species widely distributed in Belgium and France, on which in association with van den Broeck, he bestowed the name of *N. wemmelensis* ("Étude," p. 169), distinguishing the Alum Bay specimens under the name var. *Prestwichiana* (or, in the description of Plate 6, as *Prestwichi*).

In the same (posthumously published) work de la Harpe gave the name *N. elegans*, Sow., to a "species" (the megalospheric form of *N. planulata*), which, as he stated, is distinct from *wemmelensis* and its varieties, giving in the list of synonyms :—

1829, *N. elegans*, Sow. (pars) 'Mineral Conchology,' vol. 6, p. 76, Plate 538, figs. 9, 10, 11 (non figs. 6, 7, 8). Thus referring to fig. 10 among others in Sowerby's plate for an illustration of this species.

In 1886, Professor Jones having recognised that some of the specimens named *elegans* in the Sowerby Collection were identical with his *N. planulata* var. *Prestwichiana*, and de la Harpe's and van den Broeck's *wemmelensis* var. *Prestwichiana*, from Bed 29, in Alum Bay, wrote his paper on *N. elegans* and the other English Nummulites (11), in which he withdrew the name *Prestwichiana* and pointed out that the proper name of the species is *N. wemmelensis*, de la Harpe and van den Broeck, var. *elegans*, Sow. (In many parts of the paper the name *N. elegans* is, however, employed.) He also expressed the opinion that one of the sections on Sowerby's tablets was the identical specimen from which fig. 10 in Plate 538 of the 'Mineral Conchology' is taken.

It thus comes about that the same figure (Plate 538, fig. 10) in the 'Mineral Conchology' is claimed by de la Harpe as an illustration of his *N. elegans,* and by Professor Jones as an illustration of his *wemmelensis* var. *elegans* from Bed 29 of Alum Bay.

In this paper I have used the name *N. Orbignyi* (Gal.), which was given in 1837, for the species as a whole, as being that which was first applied to a member (a microspheric specimen) of the main body of it.

N. elegans, Sow. (1829) has, of course, priority over *Orbignyi* (Gal.), but this was applied to a member of the Alum Bay variety, which, according to de la Harpe, is so distinct from the Continental forms that it is impossible to take it as the type of the species (10, p. 92). These Continental forms were named *N. wemmelensis,* de la Harpe and van den Broeck in 1883.

Nummulites elegans, Sow., T. R. Jones. *Ibid.*

I have examined specimens from two localities, my own from Alum Bay and a sample from Huntingbridge. The two sets are somewhat different, and it will be convenient to deal with them separately.

Those from Alum Bay (Plate 5, *m*) are from the bed which is regarded by geologists as the bottom layer of the Barton Clay, passing conformably into the underlying Bracklesham Beds (and often referred to as Bed 29 of Prestwich's section). They are apparently identical with the specimens on the tablets 44,007 (1) and (2) of the Sowerby Collection now in the British Museum.

On grinding down 95 specimens to display the initial chambers, I find 93 to be megalospheric, and 2 to be microspheric, a proportion of 46·5 to 1.

In the *megalospheric* specimens (the *N. wemmelensis* de la Harpe and van den Broeck, var. *elegans,* Sow.) M varies (fig. 1) from 66 to 148 μ, the average being about 96 μ (Plate 5, *a–a″*).

The largest specimens of this form are about 3 mm. in the longer diameter of the test.

In the two *microspheric* specimens the value of m is 17 and 19 μ. The diameter of the test in these specimens does not exceed that of the larger megalospheric forms.*

The Huntingbridge specimens which I have examined were contained in the collection of the Sedgwick Museum at Cambridge and were kindly placed at my disposal by Professor Hughes. They were obtained by Mr. H. Keeping from this locality, which is near Fritham in the New Forest.†

On comparing these specimens with those of Alum Bay certain differences are apparent, as may be gathered from Plate 5, *a* and ***a***. In the Huntingbridge specimens the height of the chambers as seen in section (measured along the radius of the test) is less in proportion to their breadth than in those from Alum Bay. Moreover the backward slope of the septa is less marked in the Huntingbridge specimens. In both these respects it happens that these latter vary in the direction of *N. variolarius.* However, in the flatness of the test (*cf. a′* and *a′*) and in the large size of the megalospheres (fig. 1) there is no approach whatever to *variolarius.* In his "Étude" (p. 170) de la Harpe says of the species under consideration:—

---

* On looking closely at tablet 44,007 (1) in the Sowerby Collection in the British Museum, in which three specimens are represented in section, it will be found that one of them (that immediately above the yellow label) is different from the others, and with the assistance of a low power of the microscope it becomes clear that this is a section of a specimen of the microspheric form. It is in fact an example of the *N. Orbignyi* (Galeotti), the apparent absence of which from the English Beds occasioned de la Harpe's surprise.

† In his paper (4) on the Bracklesham Beds of the Isle of Wight Basin, Mr. Fisher says, p. 80, of the beds a few feet lower in the series, "the character of the matrix at Huntingbridge approaches more nearly to some of the Barton deposits than to any of the Bracklesham strata." This was written before Mr. Keeping found the nummulite bed in question, and it would appear that the presence of this characteristic fossil of the lowest Barton bed confirms the suspicion which seems to have been in Mr. Fisher's mind that he was here approaching the upper limit of the Bracklesham series.

The position of the locality is clearly indicated by the reference in Mr. Fisher's paper to the Ordnance Map. On a recent visit to the Forest, I failed to find any one who recognised it by the name of Huntingbridge, and an old keeper told me that it is now known as Three Bridges.

"Les cloisons et les chambres sont irregulières dans leur forme, leur nombre, leur épaisseur et leur inclinaison."

I have no reason to doubt, therefore, that Professor Jones was justified (11) in referring the Huntingbridge specimens to *N. wemmelensis* var. *elegans*, *i.e.*, to *N. Orbignyi* var. *elegans.*

Among 44 specimens examined in section (fig. 1) 40 are megalospheric and 4 microspheric, but this proportion is not a true indication of their relative

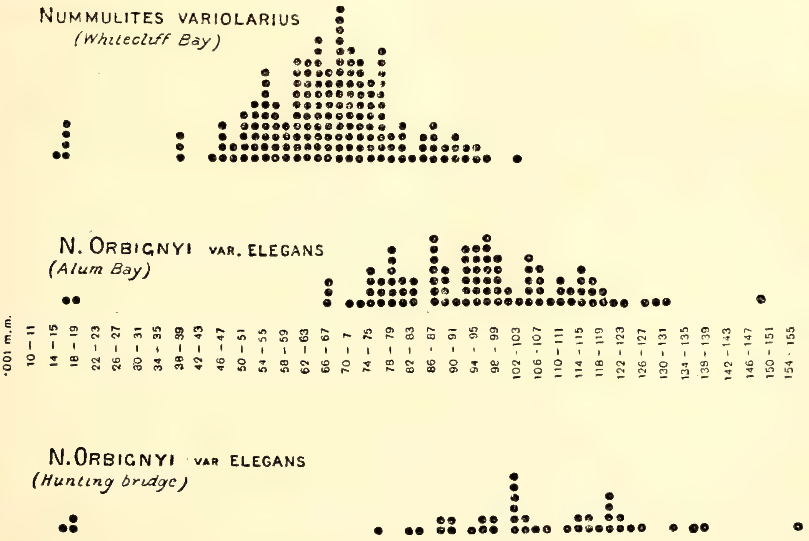


FIG. 1. Diagram showing the diameters of the Initial Chambers of the specimens measured, belonging to the species *Nummulites variolarius* and *N. Orbignyi* var. *elegans.*

Each specimen is represented by a dot. The size of each chamber (*i.e.*, the mean between its long and short diameters, as seen in the section) is indicated by the horizontal distance of the dot from zero, on the left, and may be measured by reference to the scale, indicating 1/1000th of a millimetre (μ's). The dots for chambers of the same size are piled one above another.

It will be seen that in respect of size the initial chambers of the species (or local form) fall, in each case, into two groups, the microspheric and megalospheric.

frequency in the beds because, in this case, the largest specimens are microspheric, and some of these were selected for preparing sections.

In the megalospheric examples (Plate 5, *a* and *a′*) the value of M varies from 77 to 157 μ, the average being about 106 or 10 μ greater than in the Alum Bay specimens.

The long diameter of the largest megalospheric specimen I have ground down is 2·1 mm.

In the three *microspheric* specimens in which the central chamber showed clearly enough to be measured, the value of m is 16, 19 and 19 μ (Plate 5, β'). The largest microspheric test measures 3 by 2·4 mm. There is thus in this species, though it does not happen to be shown in my Alum Bay specimens, the same tendency as was seen in *N. variolarius* for the microspheric form slightly to exceed the megalospheric in size.

It is thus evident that the English species of nummulites far from presenting exceptions to the law of distribution in pairs, are in exact agreement with that law, although the difference in the sizes of the tests between the two forms present in a stratum is, in the cases of *N. variolarius* and *Orbignyi*, little marked.

We have seen that de la Harpe himself withdrew one of his exceptions, and that two others appear to have been quoted on quite insufficient grounds; and on looking closely into his attitude towards the theory of dimorphism, it appears that his failure to admit it, and insistence on cases to which, as he supposed, it would not apply, may be attributed to the hostility which he rightly felt to an adventitious hypothesis with which that theory in its inception was for a time laden. M. Munier-Chalmas and M. Schlumberger, his colleague, recognised, at a later date, that this hypothesis was untenable, and withdrew it (17). Had he lived to reconsider the position calmly, we can hardly doubt that de la Harpe would have frankly recognised the light which the theory of dimorphism had shed on the matter.

(2) *On the Size of the Megalosphere in Relation to that of the Microspheric and Megalospheric tests.*

From the measurements given above, and also from inspection of Plates 3–5, it is clear that there is a great difference between the size of the megalosphere in *N. laevigatus* and its size in the two other species, and that these also differ in this respect from one another. From a consideration of this contrast the question arose: Is there a definite relation in size between the megalosphere, the initial chamber of the megalospheric form, and the complete test of the microspheric form?

In the life-history of *Polystomella crispa* we know that the megalospheric form arises by an asexual process; the protoplasmic contents of the microspheric form emerging from the test, and dividing up into a large number of megalospheres. These shortly separate and proceed to develop into full grown individuals of the megalospheric form (14, pp. 67 and 68).

In the light of this ascertained fact in the life-history of *Polystomella,* a simple member of the Nummulitidæ, the question under consideration may be put in a different manner: Is there a definite relation between the volume of the protoplasm of the microspheric form and the size of the megalospheres into which it divides?

For a satisfactory answer it was evidently desirable to extend the observations to a wider series of species, and, thanks to the stores of material in the collection presented to this University by the late Dr. H. B. Brady, I have been able to examine six other species (or "pairs of species" on the old view) ranging up to *N. complanata,* the microspheric form of which attains the gigantic proportions of 3 inches in diameter.

As an index of the volume of the megalosphere the cube of its mean diameter has been taken. Similarly for an index of the volume of the protoplasmic contents of the complete test the square of the diameter (d) multiplied by its greatest thickness, *i.e.*, the length of the spiral axis (a) has been taken. Thus ad^2 is the index of the volume of the complete tests, megalospheric and microspheric.

If the tests were all of the same shape and the chambers and septa between them were of the same proportional dimensions and thickness in all the species, the values of ad^2 would give accurately comparable indices of the protoplasmic contents of the tests throughout the series. This is, however, far from being the case. In some species the tests are highly biconvex, in others they are nearly flat; some have large chambers and thin walls, others small chambers and thick walls; and the species differ also in the degree of development and the disposition of the alar prolongations of the chambers. In those sometimes separated under the distinct generic name *Assilina,* the latter are altogether absent. When comparing the results the characters of each species in these respects must be taken into account.

It should be stated further that the measurements which follow can only be regarded as approximately correct. In some cases the only specimens available were glued on museum tablets, and many are more or less worn at the edges. Whenever possible the two forms of a species selected for measurement are from the same locality.

The list of species with the localities of the specimens examined is as follows:—

Nummulites complanatus, Lamk., from Hungary and Bavaria, and its megalospheric form, *N. Tchihatcheffi,* d'Arch.; from Ajka, Vesprimer Comitat, Hungary.

N. perforatus (de Montft.), Vesprimer Comitat, Hungary, and its megalospheric form, *N. Lucasanus,* Defr.; from Zircz, Vesprimer Comitat.

N. gizehensis (Forsk.) and its megalospheric form, *N. curvispirus* (Menegh); bottom of Mokattam Hill, Cairo.

N. laevigatus (Brug.) and its megalospheric form, *N. Lamarcki,* d'Arch. and Haime; from the Bracklesham Beds, Selsey.

N. biarritzensis, d'Arch., and its megalospheric form, *N. Guettardi,* d'Arch. and Haime; from Dahr el Nakhl, Egypt.

N. discorbinus (Schlot.) and its megalospheric form, *N. sub-discorbinus,* de la Harpe; from the top of the Mokattam Hill, Cairo.

N. Orbignyi (Gal.), var. *elegans,* Sow., and its megalospheric form, *N. wemmelensis,* de la Harpe and Van den Broeck, var. *elegans,* Sow.; from Barton Beds, Huntingbridge, near Fritham, Hants.

N. variolarius (Lamk.) and its microspheric form, *N. Heberti,* d'Arch. and Haime; from the Bracklesham Beds, White Cliff Bay, Isle of Wight.

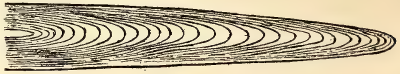
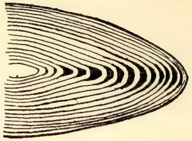
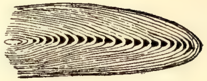
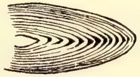
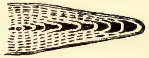
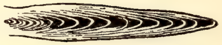
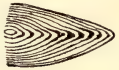
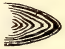
Assilina (*Nummulites*) *exponens* (Sow.) and its megalospheric form *A. mamillata* (d'Arch.); from Traunstein, Bavaria.

The results of the measurements are embodied in the following table. The species are arranged in the order of the volumes of their megalospheres. In each case, after the index of the volume of the test, the ratio which this bears to the index of the volume of the megalosphere is given.

It will be seen from the last column but one that the order of the volume of the megalospheres is, with one exception, the order of the volume of the corresponding microspheric forms.

Turning to the results entered in the last column, the ratios of the volumes of the microspheric tests to those of the corresponding megalospheres, it will be seen that although the latter range from 0·0003 to 1·161 cub. mm., the ratio is fairly constant in six cases, lying between 9000 and 12,500 to 1. Bearing in mind that it is the ratio of the volume of the protoplasmic contents of the tests to that of the megalosphere which we are endeavouring to estimate, the low figure representing *N. Orbignyi,* var. *elegans,* may not be out of harmony with these results when the characters of the species are taken into account. (Compare the large chambers and thin walls of this species as represented in Plate 5 with those of the species shown in Plates 3 and 4.)

Similarly the high figures for *N. biarritzensis* and *N. discorbinus* are swollen owing to the thickness of the shell substance which builds up their

	M.	No. measured.	M³.	Megalospheric form.					Microspheric form.					Figures representing half transverse sections of the microspheric tests, × 2.
	μ.		μ³.	Diameter (*d*).	Spiral axis. (*a*).	No. measured	*ad*².	R *ad*² : M³.	Diameter (*d*).	Spiral axis (*a*).	No. measured.	*ad*².	R *ad*² : M³.	
N. complanatus ...	1051	(12)	1·161	5·9	2·2	(19)	76·6	66 : 1	51·0	4·3	(13)	11206·2	9652 : 1	
N. perforatus	683	(7)	0·3185	5·7	2·83	(8)	91·9	288 : 1	22·1	8·0	(9)	3507·28	11011 : 1	
N. gizehensis	540	(13)	0·157	6·2	1·9	(16)	73·0	465 : 1	23·7	3·9	(12)	2190·6	13953 : 1	
N. perforatus var. *obesus*	445	(11)	0·088	3·2	1·45	(12)	14·8	169 : 1	12·1	4·1	(11)	600·3	6821 : 1	
N. laevigatus	443	(20)	0·087	4·6	1·17	(12)	24·8	285 : 1	17·2	3·45	(12)	1020·6	11731 : 1	
Assilina exponens ..	434	(6)	0·0817	8·24	1·34	(12)	90·9	1112 : 1	21·0	2·3	(6)	1014·3	12415 : 1	
N. biarritzensis ...	270	(10)	0·019	3·6	1·6	(4)	20·7	1091 : 1	11·49	4·14	(14)	547·5	27375 : 1	
N. discorbinus	174	(15)	0·005	3·04	1·65	(15)	15·2	3049 : 1	7·01	2·9	(13)	143·7	28745 : 1	
N. Orbignyi, var. *elegans* (Huntingbridge)	106	(40)	0·0012	2·1	0·47	(12)	2·07	1725 : 1	2·7	0·56	(5)	4·08	3400 : 1	
N. variolarius	68	(163)	0·0003	1·8	0·77	(13)	2·5	8333 : 1	1·92	0·82	(5)	3·02	10066 : 1	

biconvex tests without any corresponding increase in the volume of their contents.

N. perforatus, however, does appear to have a disproportionately large megalosphere, for the test of the microspheric form is nearly as biconvex as that of *biarritzensis* and *discorbinus*, yet the ratio of its volume to M^3 is not above that of the majority. The disproportion is especially marked in the case of the variety *obesus* of this species, from Beni Hassan, in which the corresponding megalosphere approaches the dimensions characteristic of the typical members of the species, although the microspheric form is much reduced.

On the whole, the results of measurement appear to indicate, for the majority of the species examined, an approximately definite numerical proportion between the volume of the contents of the microspheric test and the volume of the megalosphere. *N. perforatus* departs most widely from that proportion, and the existence of this outstanding case would render it probable that in the others the proportion, if it could be measured exactly, would be found to be only approximately uniform.

In the account given above of the mode of origin of the megalospheric form of *Polystomella crispa*, the parent organism is described as microspheric. This is in accordance with a large series of observations on this species (13, p. 446), and I am not aware of any direct evidence showing a departure from the rule in *Polystomella*.

In other orders of foraminifera, however, viz., in *Cornuspira*, *Miliolina*, *Peneroplis* and *Orbitolites* among the *Miliolidæ*, and in *Cristellaria* (1, Plate 68, fig. 1, and 14, p. 116) among the *Lagenidæ*, it is the fact that the megalospheric form is capable of repeating itself, by giving rise to a brood of megalospheric young.

It appears to me that the approximately close relation which has now been shown to exist between the size of the megalosphere and that of the microspheric form is an indication (though it cannot be considered a proof) that this latter mode of reproduction did not occur among the nummulites of the Eocene Period, as it does not occur (so far as the evidence goes) in their ally, *Polystomella*, at the present day. For if the small megalospheric forms of the species in which microspheric forms attain so large a size were thus to reproduce their like, there seems no reason why the megalospheres thus produced should be of a size proportional to the volume of the microspheric forms.

In the account given in the first part of this paper of the three English species, it will be noticed that though the megalospheres attain very different diameters the microspheres are remarkably uniform in size, all falling between 15 and 19 μ in diameter. In most of the other species which I have examined in section, the calcite filling of the tests obscures the central chambers so much that it is rarely possible to obtain an accurate measurement of a body so minute as the microsphere. However, in one specimen of *N. gizehensis* this can be done, and the value of *m* corresponds closely with that found in the three English species, being 20 μ. From these few cases (and I am not aware of any other records of the actual size of the microsphere in this genus) it would appear that the size of the microsphere is independent alike of the size of the megalospheric form and of the ultimate size of the microspheric test into which it grows.

In view of the facts (*a*) that there is good ground for concluding that the microsphere arises by the conjugation of zoospores produced by individuals of the megalospheric form, and (*b*) that the megalosphere arises by an asexual process, the results so far obtained may be stated for the genus *Nummulites* as follows:—

The size of the asexually produced megalosphere is approximately proportional to the volume of the protoplasmic contents of the microspheric parent.

The size of the (probably) sexually produced microsphere is uniform, or nearly so, throughout.

Turning now to the eighth column in the table, showing the ratios of the volume of the megalospheric tests to that of the megalospheres, it will be noticed that as the series of species is followed down, the ratios increase, though with some irregularity, from the top to the bottom. In the little *N. variolarius*, this ratio is almost the same as that for the microspheric form—the tests of the two forms being, as we have seen, almost of the same size. In the other species as the volume of the microspheric form increases so does the proportion which the volume of the megalospheric form bears to the megalosphere decrease.

In the case of *variolarius*, as the tests of the two forms attain about equal sizes, we may suppose that each took about the same time to grow, or, in other words, that the complete cycle of the life-history was divided in two nearly equal parts between the alternating generations. But in the other species it would appear that in proportion as the period of growth of the microspheric form preponderated in the life history, so did that of the megalospheric form diminish—not only in proportion to the microspheric form of the same species, but in proportion also (allowing for the difference in volume) to the period of growth of the megalospheric form in a species such as *variolarius*. Thus, to compare this species with *N. complanatus*, its megalospheric form, when full grown, attains a size (test included) over 8000 times as large as that of the initial chamber in which it began. In *complanatus* the proportion of these volumes is only 66 to 1.

In the approach to equality in the sizes of the tests of the two forms the species *N. Orbignyi* and *variolarius*, though exceptional among nummulites, agree with the majority of the foraminifera in which dimorphism has been recognised. The genus *Polystomella* is an example among the *Nummulitidæ*, in which the two forms are also of equal size. On the other hand, in *Heterostegina*, probably in *Cycloclypeus* and in at any rate several of the species of *Orbitoides*,* the microspheric form preponderates over the megalospheric, as it does in most of the nummulites. Outside the *Nummulitidæ* we meet with the preponderance of the microspheric form in certain genera of the *Miliolidæ* (*Biloculina*, *Miliolina*, and in *Orbitolites complanata*), and it would be interesting to learn how far a similar correspondence in size between the microspheric form and the megalosphere obtains in these cases, and whether the repetition of the megalospheric generation produces any modification of the results.

These stages in the reduction of the megalospheric or gamete-producing generation are interesting from a wider biological standpoint as affording a parallel with what has occurred in other groups of animals and plants. Thus, to take a particular instance, we may compare the small and short-lived *Nummulites Tchihatcheffi*, a dwarf beside the great disc of *N. complanatus*, with the prothallus of a fern, arising asexually from a spore, and ultimately producing a zygote (by the union of gametes) which grows into the long-lived and comparatively gigantic sporophyte.

When the nuclear history of the foraminifera comes to be more perfectly known, it will be interesting to learn how far it runs parallel in two so widely separated forms. At present our knowledge of it is too incomplete to allow a comparison to be profitably instituted.

While writing the first part of this paper, I was beset by a suspicion that I was perhaps making too much of the difficulties raised by de la Harpe, that everyone who is interested in the life-history of the foraminifera was convinced long ago of the general prevalence of dimorphism, and that I might therefore have set out to fight the already slain.

That this is not the case is shown by a memoir which has just reached me by MM. Lemoine and R. Douvillé, *Sur le genre Lepidocyclina* (12), a copy of which I owe to the courtesy of the authors. Written as this memoir has been in close association with M. Schlumberger, who in the fine series of papers on the structure of the tests of the *Miliolidæ* and other forms has done so much, either alone or in conjunction with M. Munier-Chalmas, to establish the dimorphic character of the tests of the species of foraminifera, the views herein expressed carry the greater weight. On p. 7 we read, under the heading Dimorphisme:—

---

* See the series of papers published by M. Schlumberger on the subgeneric groups *Orbitoides*, *Orthophragmina*, and *Miogypsina* (19) and the memoir by MM. Lemoine and R. Douvillé on *Lepidocyclina* (12).

"Chez les Orbitoïdes, comme chez la plupart des Foraminifères on trouve deux séries, une forme A à mégasphère, une forme B à microsphère. On sait maintenant par les observations de Schaudinn et par celles de Lister que ce dimorphisme est dû *dans certains cas** à un phénomène de génération alternante. La forme mégasphérique donne, par sporulation, des zoospores; ces zoospores se conjuguent, et le produit de leur conjugaison, en se développant, donne naissance à la forme microsphérique. Celle-ci par bourgeonnement redonne des formes mégasphériques.

"Le cycle n'a d'ailleurs jamais été suivi complètement. Il est très probable que les formes mégasphériques ou microsphériques peuvent se reproduire directement l'une ou l'autre, sans passer par la forme alternée. Nous ne savons rien des conditions de milieu que déterminent la prédominance partielle ou totale de l'une ou l'autre de ces formes; mais, ce qui est certain, c'est que ces deux formes couplées n'ont qu'exceptionnellement la même extension verticale."

This last passage not only emphasises the view temporarily held, and, as we have seen, abandoned by de la Harpe, of the occurrence of solitary forms in certain beds, but goes far to undermine his law of the association of species, for which there is such abundant evidence.

From the facts of the life-history now known to us, such a phenomenon as the authors describe would mean, if we are to take the vertical distribution of the species in every case as the unaltered record of the phenomena exhibited by it during the period in which it inhabited a given locality, that either at the beginning or the end of that period, or at both, only one phase was present in the life-history, while at another part of that period the species became or had been dimorphic.

But the phenomenon was, I believe, well known to Munier-Chalmas and attributed by him to a redistribution of the materials of a bed under the action of currents—the coarser fragments being deposited here and the finer elsewhere. And until such an explanation has been excluded it is surely unnecessary to introduce so complex and incomprehensible a conclusion into our ideas on the life-history.

---

* The italics are the authors'.

That the megalospheric form in some families may reproduce its like for one, and very possibly for a series of generations, is well known, but where is the evidence that the microspheric form ever reproduces the microspheric directly? I have never seen or read of one particle of evidence to this effect. If dimorphism is due "*in certain cases*" to alternation of generations, to what other mode of life-history or cause of any kind is it to be attributed in closely allied forms?

A little further on the authors cite a paper (8A) by M. E. Haug, in which he simply records the fact that, among a number of examples of *N. variolarius* in a certain bed, he did not find the microspheric form (*N. Heberti*); though he is far from drawing the conclusion, which the authors are inclined to draw, that it was, in fact, absent. De la Harpe found, as we have seen, that it is impossible to distinguish this form from the megalospheric, by examination of whole specimens, and in my own experience it was not till I had ground down 45 specimens of this species, all of which proved to be megalospheric, that I happened on an example of the microspheric form. I cannot admit, therefore, that such negative evidence, unless supported by observations on the size of the initial chambers of a large number of specimens, has any force to shake a conclusion so firmly established.

If I put these objections abruptly, I beg the authors to believe that I do so in no hostile spirit, but for the furtherance of the subject in which we are alike interested. It seems to me that we are in danger of letting drop the clue which is within our grasp.

Summary.

The results obtained in this investigation may be summarised as follows:—

1. Both microspheric and megalospheric forms of *N. variolarius* and *N. Orbignyi* var. *elegans* are present in the Eocene Beds of the Isle of Wight and Hampshire, as I believe they will be found to be present elsewhere, except when the materials of a bed have been rearranged under the influence of currents.

2. In these species and in *N. laevigatus* and *N. gizehensis* the size of the microsphere is nearly constant—the diameters in the specimens measured being between 15 and 20 μ.

3. In the nine species and one variety of *Nummulites* and *Assilina* which I have examined, the size of the megalosphere is approximately proportional to the volume of the contents of the microspheric form.

By this result additional proof is given of de la Harpe's conclusion, founded on the mode of occurrence in the beds, and on structural features of the tests of the two forms, that these are in each case truly members of "a pair," or, as we now say, are related as alternating or recurring forms in the life-history of a species.

By (2) and (3) the two modes of reproduction come into marked contrast: the asexually produced megalospheres being approximately proportional in size to the protoplasmic volume of the parent, while the microsphere, probably arising as a zygote, is uniformly small throughout.

4. In several of the species examined, as the microspheric member of the cycle preponderates in the life-history, the megalospheric member decreases, not only in proportion to the size of the microspheric form, but in proportion to the megalospheric members of other species in which the two forms attain approximately equal sizes.

In conclusion, I wish to express my thanks to Dr. Harmer, the Superintendent of the Museum of Zoology in this University, for the free use he has allowed me to make of the ample stores of material in the Brady Collection; and to Professor Hughes for kindly placing at my disposal the specimens of *N. Orbignyi* var. *elegans* from Huntingbridge, contained in the Sedgwick Museum; also to Mr. H. Keeping of that Museum, whose knowledge of the beds of the Hampshire Basin has considerably assisted me.

I have to acknowledge the helpful advice I have received from my friend Mr. A. Harker in preparing the table on p. 311. To Mr. R. B. Newton, of the British Museum, I am also indebted for his assistance when examining the specimens in the Sowerby Collection there contained.

The photographs with which this paper is illustrated were made with the kind help of my brother, Mr. W. T. Lister. They were done with the excellent Zeiss instrument in his possession.

LITERATURE QUOTED.

1. Brady, H. B., Report on the Foraminifera collected by H.M.S. "Challenger" (1884).
2. Van den Broeck, E., "Comment faut-il nommer les Nummulites, en tenant compte de leur dimorphisme?" 'Bull. Soc. Belge d. l. Géologie,' vol. 10, fasc. 4 (1896), p. 50.
3. Douvillé, H., "Études sur les Nummulites, 1re Note," 'Bull. Soc. Géol. de France,' 4 sér., vol. 2 (1902), p. 207.
4. Fisher, O., "On the Bracklesham Beds of the Isle of Wight Basin," 'Q. J. Geol. Soc.,' vol. 18 (1861–2), p. 65.
5. De la Harpe, Ph., "Étude des Nummulites de la Suisse, et revision des espèces Éocènes des genres *Nummulites* et *Assilina*," 'Mém. Soc. Paléont. Suisse,' vols. 7–10, 1881–3.

6. De la Harpe, "Sur l'importance de la loge centrale chez les Nummulites," 'Bull. Soc. Géol. de France,' sér. 3, vol. 9 (1881), p. 171.
7. De la Harpe, "Monographie der in Aegypten und der Libyschen Wüste vorkommenden Nummuliten," 'Palæontographica,' vol. 30 (3 Folge, Bd. 6), p. 155.
8. De la Harpe, "Note sur la distribution par couples des Nummulites Éocènes," 'Bull. Soc. Vaudoise des Sciences naturelles,' sér. 2, vol. 17 (1881), p. 429.
8A. Haug, E., "Sur l'âge des couches à *Nummulites contortus* et *Cer. Diaboli*," 'Bull. Soc. Géol. de France' (4), vol. 2 (1902), p. 483.
9. Jones, T. Rupert, "Note on *Nummulina planulata*, Lamarck, sp. var. *Prestwichiana*, Jones," Appendix B to O. Fisher's paper, q.v. (4).
10. Jones, T. Rupert, "Catalogue of the Fossil Foraminifera in the British Museum," London, 1882.
11. Jones, T. Rupert, "On *Nummulites elegans*, Sowerby, and other English Nummulites," 'Q. J. Geol. Soc.,' vol. 43 (1887), p. 132.
12. Lemoine, P., and Douvillé, R., "Sur le genre *Lepidocyclina* Gümbel," 'Mém. d. l. Soc. Géol. de France,' No. 32, t. 12, fasc. 2 (1904).
13. Lister, J. J., "Contributions to the Life-History of the Foraminifera," 'Phil. Trans., vol. 186, B (1895), p. 401.
14. Lister, J. J., Article, "*Foraminifera*," in Lankester's 'Treatise on Zoology,' Part I. fasc. 2, London, 1903.
15. Munier-Chalmas, "Sur le Dimorphisme des Nummulites," 'Bull. Soc. Géol. de France,' ser. 3, vol. 8 (1880), p. 300.
16. Schaudinn, F., "Ueber den Dimorphismus der Foraminiferen," 'Sitz. Ber. d. Gesellsch. naturforsch. Freunde zu Berlin,' 1895, No. 5.
17. Schlumberger, C., "Note sur la Biologie des Foraminifères," 'La Feuille des Jeunes Naturalistes' (3), An. 26 (1896), p. 305.
18. Schlumberger, C., "Note sur le genre *Miogypsina*," 'Bull. Soc. Géol. de France' (3), vol. 28 (1900), p. 327.
19. Schlumberger, C., "Notes sur les Orbitoides," *ibid.* (4), vols. 1–4 (1901–4)
20. Sowerby, J. de C., 'Mineral Conchology,' (vol. 6, 1829).

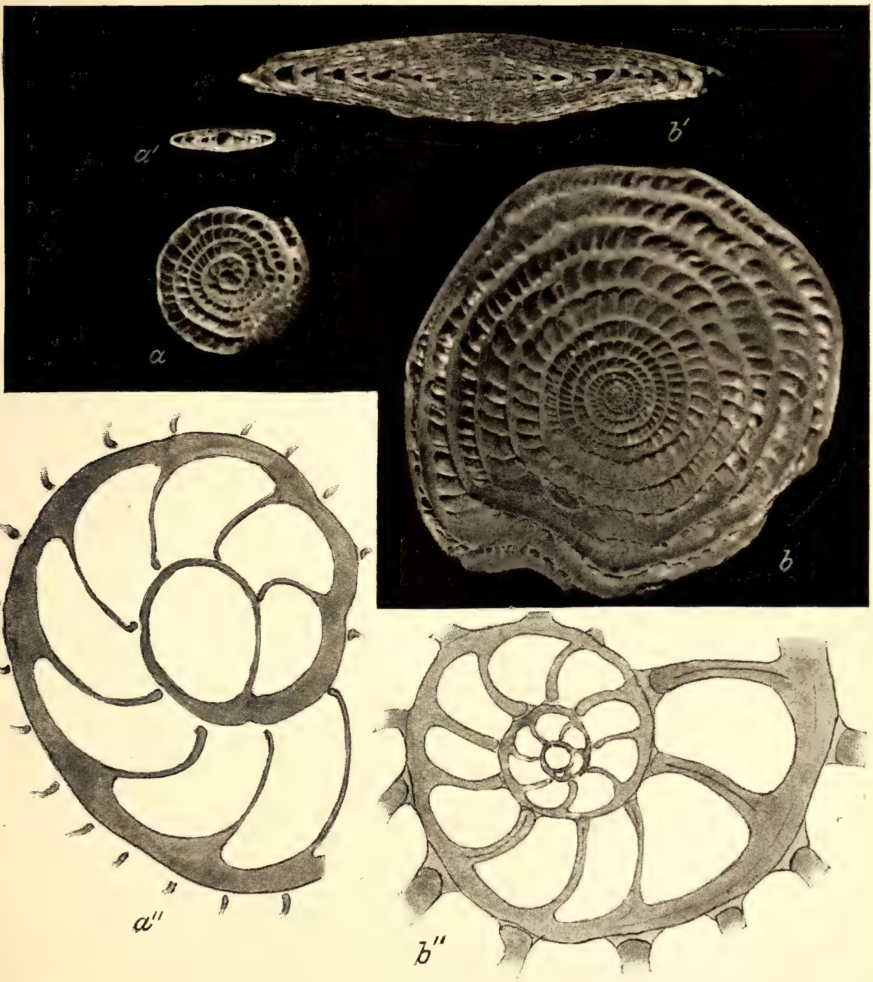
DESCRIPTION OF PLATES.

PLATE 3. *Nummulites laevigatus* (Brug.).

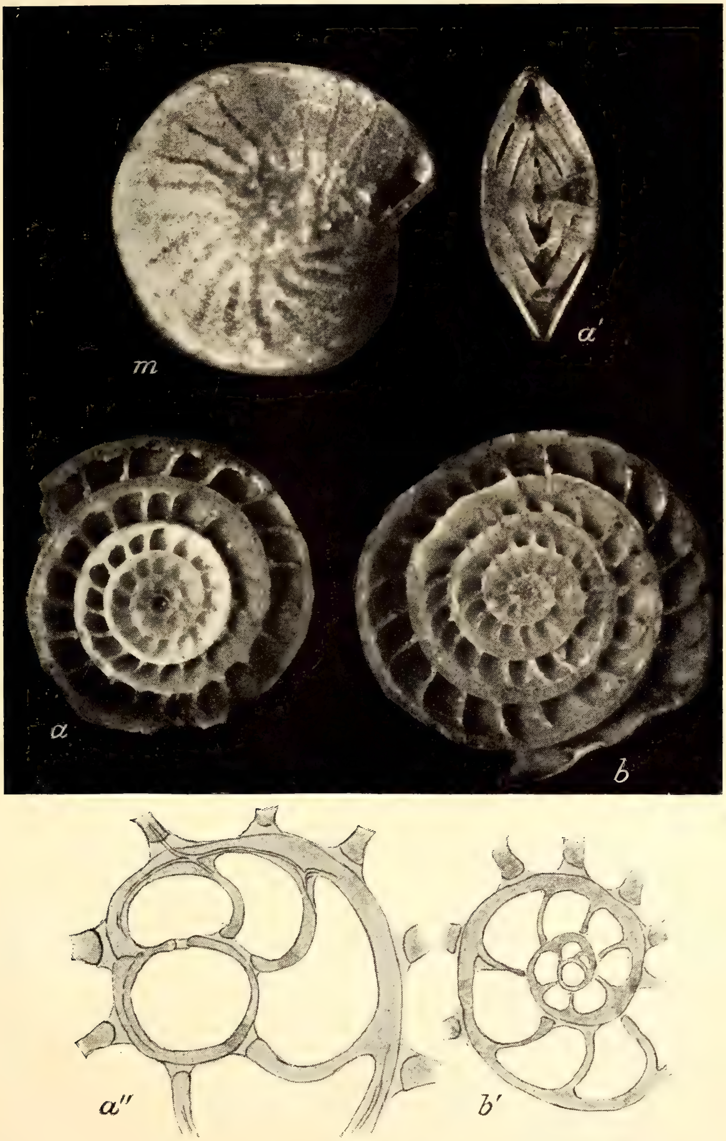
a and *a′*, median and transverse sections of the megalospheric form (= *N. Lamarcki*, d'Arch. and Haime).
b and *b′*, median and transverse sections of the microspheric form. All × 4.
a″, central region of a median section of the megalospheric form. × 38.
b″, central region of a median section of the microspheric form. × 200.

PLATE 4. *Nummulites variolarius* (Lamk.).

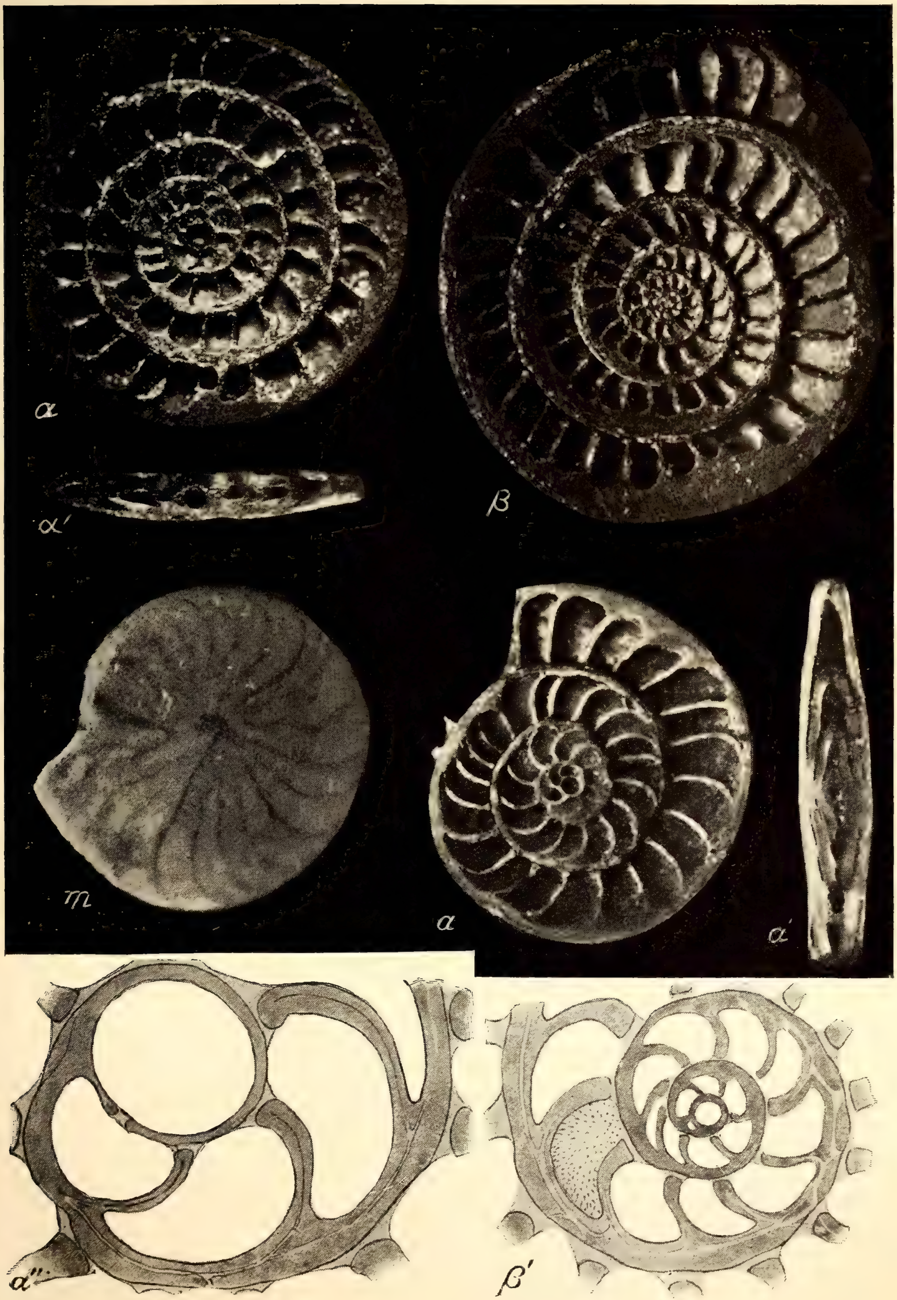
m, side view of the test; *a* and *a′*, median and transverse sections of specimens of the megalospheric form; *b*, median section of a specimen of the microspheric form (= *N. Heberti*, d'Arch.). All × 24.
a″ and *b′*, central regions of sections of the megalospheric and microspheric forms. × 200.



Nummulites laevigatus (Brug.).
(*N. Lamarcki*, D'A. and H.).



Nummulites variolarius (Lamk.).
(*N. Heberti*, D'A. and H.).



Nummulites Orbignyi (Gal.).
(*N. wemmelensis*, D.L.H. and V.D.B., var. *elegans*, Sow.).

PLATE 5. *Nummulites Orbignyi*, Gal. (*wemmelensis*, de la Harpe and van den Broeck), var. *elegans*, Sow.

m, side view of a specimen from Alum Bay; *a* and *a′*, median and transverse sections of specimens of the megalospheric form, from Alum Bay; *a* and *a′*, median and transverse sections of specimens of this form from Huntingbridge; *β*, median section of a specimen of the microspheric form (= the *N. Orbignyi* of Galeotti), from Huntingbridge. All × 24.

a″ and *β′*, central regions of sections of the megalospheric and microspheric forms (*a″* from Alum Bay, *β′* from Huntingbridge). × 200.

In Plates 3 *b″*, 4 *a″*, and 5 *a″* and *β′*, some indication of the presence of the canal system is given, and in 5 *β′*, the further side of one chamber is drawn to show the perforations in the wall. The perforations are not present in the median planes of the tests.

On the Occurrence of Anopheles (Myzomyia) Listoni *in Calcutta.*

By J. R. ADIE, M.B. (Lond.), Major, I.M.S., and A. ALCOCK, M.B., LL.D., F.R.S., Major, I.M.S.

(Received April 15,—Read May 18, 1905.)

Stephens and Christophers, in their original report* to the Royal Society, on the Relation of Malarial Endemicity to Species of *Anopheles*, state, as their second conclusion, "that the distribution of *A. Christophersi* [= *Myzomyia Listoni*] corresponds closely with an area of extremely high endemicity."

It will be remembered that they were discussing the relation of malarial endemia to species of *Anopheles* in Bengal; that they found this endemia to vary from 0 in Calcutta to 7 in Barrackpore, 12 in Jalpaigori, and 72 in the Duars (increasing as they proceeded north); and that they concluded "that the distribution of certain species coincided with areas of high endemicity, while other species occurred, and even existed in profusion, where very little infection was present."

All this is graphically illustrated in their well-known "Sketch Map" (reproduced in their new book) which shows the Calcutta region marked *Rossii*, a non-malarial carrier, and the Duars marked *A. Christophersi* [= *M. Listoni*] an undoubtedly good carrier.

Stephens and Christophers were careful to say that their observations relating to distribution and endemia in Bengal were conducted in June, July, and August, and though they came to the general conclusions quoted above they insisted that much more extended observations were required.

---

* Malaria Reports, Sixth Series, March 6, 1902.

In the second edition of their book,* just published, this generalisation is repeated—"We may have countless numbers of *M. Rossi* as in Calcutta (environs) and get a malarial index of 0, and this appears to hold good in Madras, Bombay, and, as far as our observations go, universally. On the other hand, where we find *M. Listoni, M. culicifacies, P. Jeyporensis,* in India we have a high endemic index."†

This preface is necessary in order to realise the importance of the discovery, lately, of *M. Listoni* in Calcutta.

During the last Christmas holidays we found, in the Museum tank in Calcutta, considerable numbers of *Anopheles'* larvæ, which were immediately examined, and referred to the *nigerrimus* and *culicifacies* groups. On breeding out, these were found to be *M. nigerrimus* and *M. Listoni,* the former being much more numerous. The larvæ were found in shady spots near the bank, chiefly near and under a raft. There was abundant aquatic vegetation and fish (*Cyprinidæ, Saccobranchus,* and *Ophiocephalus*) are numerous; it was, in short, an ordinary Calcutta tank. Our specimens show very well the palpal bands as described by Stephens and Christophers, "palpi, two broad apical bands . . . . . one narrow basal."‡

This capture, in the first place, extends the geographical distribution of this species given in the books. It is the only malaria-carrier that has been reported from Calcutta, other *Anopheles* being *Rossi, fuliginosus, nigerrimus, Stephensi.* In the next place it seems to show—as, indeed, would be expected *a priori*—that generalisations as to seasonal prevalence of specific *Anopheles* and endemic indices deduced therefrom, can only be made with confidence when observations are carried on throughout the year.

"Endemic index" is described as the percentage of infected children in any district, and in the investigations of the Malaria Commission the figure 0 for Calcutta was arrived at by the examination of only 191 children in June, July, and August, and was regarded as confirmed by the absence during those months of all the known malaria-carriers.

Now, however, that the approved malaria-carrier *Anopheles* (*Myzomyia*) *Listoni* is known to be present in Calcutta in December and January (none have been found in February) it seems reasonable to suppose that the June—August observations may have merely coincided with the trough of a then dormant endemic curve for Calcutta. This would be agreeable to clinical experience, for the statement that there is no endemic malaria in Calcutta was a blow to many medical men in that city.

---

* 'Practical Study of Malaria,' p. 258.

† See also Malaria Reports, Series VII, p. 23.

‡ P. 195, new edit.

Incidentally we should like to put on record, as an outcome of the present report and as a suggestion for other observers, the results of an experiment undertaken to discover the natural foes of *Anopheles'* larvæ, about which, it seems, more is taken on hearsay than is actually known.

On February 7 three sets of larvæ of all ages were placed, under conditions as natural as possible, in three separate cages. In the first cage there were 12 larvæ in water from which other aquatic animals had been strained. From these 4 adults hatched out. In the second cage there were 14 larvæ in "strained" water. From these 5 adults hatched out. In the third cage there were 12 larvæ and a single rapacious larva of a dragon-fly (the common *Ceriagrion coromandelianus*). In this cage all the *Anopheles'* larvæ disappeared, and on February 22 an adult dragon-fly hatched out.

The Structure and Function of Nerve Fibres.—Preliminary Communication.

By J. S. MACDONALD, Professor of Physiology in the University College, Sheffield.

(Communicated by Professor C. S. Sherrington, F.R.S. Received April 17,—Read May 18, 1905.)

The electrical phenomena characteristic of nervous function have always served to attract attention to the distribution of electrolytes within nerve-fibres. The complexity of this distribution has been realised since the early observations of Du Bois Reymond, who first clearly defined the necessity of making all available information of this kind the basis from which to discuss the electrical phenomena elicited from nerves. An important hindrance to the acquisition of the necessary detail is offered by the extreme minuteness of the individual nerve-fibre. Most of the information already acquired has therefore of necessity been indirectly obtained, and can in general be described as a series of deductions drawn from a detailed observation of peculiarities in the conductivity of the nerve-trunk—a structure containing some thousands of nerve-fibres arranged in a complex of ensheathing tissues. It has been found that the conductivity of the nerve-trunk cannot be expressed as that of a simple homogeneous conductor, and can only be imitated by an arrangement of at least three kinds of conducting material in a definite pattern—an external medium of poor conductivity, a dividing membrane, and an internal solution of a conductivity of a higher order than that of the external solution. With good reason the properties of these three hypothetical tissues have usually been transferred to the structures of the individual nerve-fibre. For example, it has been frequently supposed that its axis-cylinder represented the internal solution of more than usual conductivity.

Within recent years evidence has been gradually collected pointing to the fact that the major part of the conductivity of the tissues of the body, nerve included, is due to the inorganic salts they contain. It is therefore a fair assumption that here, where the internal solution of the nerve has been credited with an unusually great conductivity, this greater conductivity is due to the presence of an unusual concentration of inorganic salts within the nerve-fibre. Unfortunately, this statement cannot be adequately tested by an appeal to the "ash" of the nerve-trunk, since the axis-cylinders of the nerve-fibres form only a small proportion of the total bulk thus examined.

The only fact gleaned from such an examination is that the salts present are very largely salts of potassium. Summing up all this indirectly-obtained evidence, it might therefore be said that there was reason to believe that a solution of potassium salts of more than usual concentration was present in the interior of individual nerve-fibres.

Appreciating the fact that this statement might be critically examined in its bearing upon any one of the electrical phenomena elicited from the nerve, I made, a few years ago, a careful and detailed inquiry into the phenomenon known as the injury-current. Taking the supposed greater conductivity of the internal solution as a main guide to the choice of appropriate methods of experiment, I examined the possibility that the injury-current was due to the diffusion from this more concentrated solution first permitted by the circumstances of injury. Electrodes placed upon the transverse-cut end and the longitudinal surface respectively were treated theoretically as if the first was in contact with the internal solution, the second in contact with the external solution, of a hypothetical single nerve-fibre represented by the nerve-trunk. Modifying the value of the external solution, I found that a very precise modification of the value of the electromotive force thus measured followed each variation in the external solution. The relation thus found between the value of the external solution and that of the electrical phenomenon I called the "Concentration Law."[3] Its nature was such as to confirm the opinion that in this arrangement I was dealing with a liquid concentration cell, but if so it was evidently a special case in which the conditions present were curiously simple. Judging from the numerical statement of the law alone, this simplicity indicated a relation between the solutions present such that the internal solution must be supposed to be ten times more concentrated than the external solution. Such a difference of concentration as this seemed on other grounds improbable, and the necessity for such a conclusion entails a critical examination of the kind of concentration cell supposed to be thus formed and examined in the nerve. The hypothetical contents of this cell are (1) the internal solution, (2) a membrane of undetermined but presumably limited permeability, (3) the external solution. Knowing nothing as to the probable effect of the interposition of this membrane, it seemed best to seek for the case in which the value of the cell was reduced to zero. In this case it seemed fair to infer that the peculiarities of the membrane, supposing its permeability to be the same in both directions, were eliminated, and that the cell contents were now: (1) a solution, (2) a membrane, (3) another solution similar to the first. Having sought experimentally for such instances,[1] and

---

NOTE.—[3], etc. These numbers refer to the entries in the list of references printed at end of paper.

for instances in which the value of the cell was reversed, and found them, the general conclusion arrived at remained as before. The hypothesis being correct, the value of the internal solution was ten times greater than that of the decinormal solution (lymph) found bathing the outer surface of the nerve.

This conclusion necessarily brought me up against the facts of osmotic pressure. I therefore studied the alterations in volume of the nerve consequent upon alterations in the strength of the external solution,[2] and failed to find in them any support for the idea that there was an extraordinarily concentrated solution within the nerve-fibre. Obviously, as such experiments at once show, the osmotic pressure exerted by the internal solution of the nerve is similar to that of the decinormal solution found upon its surface. Still, believing that the conductivity of the internal solution was of a higher order than that of the external solution, and that the injury-current was in reality produced by a diffusion process between the two solutions, I was, however, forced to the conclusion that the major part of the inorganic salts within the nerve-fibre were arranged there in some special manner.[1] It was necessary to assume that the salts only possessed the osmotic pressure due to their real quantity, and were only capable of taking part in processes of diffusion in this real quantity, at the actual site of injury. It was not possible to consider that they were elsewhere in a state of ordinary chemical union, since they played their proper part in the conductivity of the nerve. In my imagination they were arranged along hypothetical surfaces, the surfaces of the fibrils, in such a way that they were free to move along these surfaces, but not at right angles to them. Subsequent microscopical experience has convinced me of the artificial character of these neuro-fibrils, and I am now logically driven to consider the concealed salts as present upon the surfaces of particles existing in the colloidal solution of the axis-cylinder.

Within recent years Macallum, of Toronto, has considerably extended the methods available for the direct observation of the chemical constituents packed away within the minute and enclosed cellular elements of the tissues, and has placed at our dispcsal a number of reactions which can be watched beneath high powers of the microscope. In his own hands and in the hands of others who have adopted them these methods have already led to the collection of a mass of most interesting data. Most recent of these methods has been one for the detection of potassium salts.[7] Using this method—precipitation with cobalt nitrite, and subsequent blackening of the precipitate by the addition of ammonium sulphide—Macallum has carefully examined the case of the nerve-fibre. His observations have led him to certain conclusions as much opposed to the evidence obtained from osmotic-pressure experiments as were mine. Macallum's conclusions, however, are entirely in the opposite direction. He has come to the conclusion that, except in certain limited spots, there are practically no electrolytes present in the axis-cylinder of the nerve-fibre. This finding is, indeed, not only opposed to the teaching of osmotic-pressure experiments, but also to that of all the very numerous series of experiments in which the peculiarities of the electrical conductivity of nerves have been studied. His conclusion is based upon the fact that the reagent used has only precipitated potassium salts at certain points—nodes of Ranvier, and certain indefinitely placed intermediate points—which taken together probably do not represent more than one-hundredth part of the total length of the nerve-fibres.

The pith of this contribution is the fact that I have to record a series of observations, which have amply served to substantiate my original conclusion and to refute Macallum's. These observations show that it is true that a solution of inorganic salts, neutral salts, is present at every site of injury to a nerve-fibre of a strength not to be found in the same way in other parts of the fibre. Such a solution can be observed at any and every point of a nerve-fibre by the simple resort of injuring that part of the fibre. The evidence upon which this statement is based is histological in character, and has been obtained by two methods of observation. The first method has been the examination of nerve-fibres in a dye,[5] neutral red, extremely insoluble in, and therefore precipitated by, neutral inorganic salts. The second method has been the use of Macallum's own reagent. I will begin by considering the appearances to be observed in the nerve-fibre, when examined in solutions of neutral red.

Neutral red stains the nerve-fibre a uniform pink colour, sometimes an orange-yellow. Presumably these slight differences mark slight modifications in the relative amount of acid and alkaline salts present, as the dye is a very sensitive indicator. In addition to this uniform staining of the fibre, certain points are peculiarly affected by the dye: (1) the sites of injury; (2) some of the nodes of Ranvier; (3) and certain granules which form first in the situations already given, but which finally spread from thence, involving very considerable stretches of the nerve-fibre. At these special points there is a very intense collection of the stain; under certain conditions red, under other conditions yellow. Apparently, therefore, the sites of injury may be slightly acid (acid salts) or slightly alkaline (alkaline salts). Whether slightly acid or slightly alkaline, it is in both cases possible to prove, under the microscope, the presence of the injury-current. There can, therefore, be no longer any room for the contention that this current is a consequence of an output of acid waste-products originating from some gross chemical change in the proteids of the axis-cylinder. The presence of the injury-current in fibres still under observation with the microscope can be determined by a resultant formation of deeply stained granules, the granules mentioned above. These granules are not structures pre-existent in the nerve-fibre that are successively stained by the gradually invading dye. They are first observed at a time when the dye has already reached situations far in advance of the site of their formation. They may be seen to grow from scarcely visible points into irregular spherules of considerable size. That they are not formed by any uncomplicated process of diffusion is deduced from the rate with which their successive appearance involves measured lengths of the axis-cylinder. Equal lengths of the axis-cylinder are involved in exactly equal times. The invading material giving rise to their formation advances into the axis-cylinder, therefore, at an absolutely uniform rate. The rate of advance is of the same order as that of known ionic velocities.[5] The inference is, therefore, drawn that they represent the consequences of the conduction of the electrical current along a perfectly insulated path. The material giving rise to their formation comes from the site of injury and not from outside of the fibre, and is probably the material, which at the site of injury forms the newly deposited cause of the injury-current. Modifying the probable value of the injury E.M.F. by my original expedient of varying the external solution—changing the value of the salt solution in which the dye is presented to the fibres—the rate of granule formation undergoes anticipated changes. Within the small limits of modification practicable, the rate is inversely proportional to the strength of this outer solution. It should be noted that there is some difference between the behaviour of these granules[6] when reddened by the stain from that of those observed under conditions such that they are yellow. The yellow granules disappear with much greater rapidity, leaving no trace of their former presence. The conditions leading to their appearance are, it may be concluded, therefore, readily reversible, since the accumulation of material at the site of injury and at neighbouring points vanishes in this case more rapidly than can be explained by its dispersal along the nerve-fibre. The inference is drawn that this material reassumes the original relation to the colloidal solution of the nerve-fibre which it possessed previous to the occurrence of injury. The reddened granules are more lasting, although not permanent. They are, therefore, more easily observed, and the definite statements made as to the rate of granule formation are made from the results of their observation. In the nerve-fibres of cooled frogs the granules are yellow, in those of warmed frogs they are yellow.

The appearances seen at the nodes of Ranvier have yet to be considered. It is conceivable that the nodes of Ranvier may represent situations easily injured by such stretching of the fibre as is incidental to the process of teasing. This supposition may account for the appearances seen at some few of the nodes, but not for the greater number of those observed. The only nodes consistently affected in every preparation are such as belong to internodes in which there is, usually at some distance, some other site of injury obviously present—for example, the nodes next to the cut ends of the fibres. The large majority of the other nodes escape. Again, it is very notable that such nodes as are affected are involved at a much later time than the ordinary sites of injury. It therefore seems possible that the staining effects at the nodes are due not to primary but to secondary accumulations of material deposited during the progress of the injury-current. I have previously stated my inference that the internodal segments of the fibre represent a perfectly insulated path, that is to say, that the myelin-sheath is perfectly impermeable to the ions carrying the injury-current. The nodes of Ranvier are therefore left as the only situations through which the current can leak. It is possible that, although relatively permeable, they are still not absolutely permeable. They would then form the site of secondary accumulations of material in transit, and, therefore, of processes of polarisation. Digressing for a moment to consider the bearing of such a statement upon the polarisation of non-medullated nerve. It is conceivable that these fibres, possessing no nodes of Ranvier, are not so perfectly insulated as medullated nerves which do, since the non-medullated nerve is one long node of Ranvier throughout its whole length.

It will be seen later that all the appearances I have described are due to the deposition of potassium salts at the site of injury, and their migration from thence with the injury-current. I have now, however, to relate something of the difficulty into which I got in attempting to prove this fact previous to the great assistance provided by Macallum's reagent. Before doing so, however, I may be allowed to sum up the appearances described above in terms of this statement, that they are produced by precipitations of dye in solutions of potassium salts. At the site of injury potassium salts are found in concentrated aqueous solution. The process giving origin to their appearance is a reversible process, they may disappear from this state of simple aqueous solution, returning to their original concealed condition. From the site of injury this potassium salt migrates at a uniform pace into the nerve-fibre. Secondary accumulations of potassium salts occur at the nodes of Ranvier.

Having observed results presumably due to the differential precipitation of the dye in different parts of the nerve-fibre, I proceeded to study more minutely the manner in which the dye is actually thrown down from its solution in water by the addition of varied quantities of potassium salts—examining the precipitates under the same powers of the microscope as had been used in the study of the nerve-fibre. Precipitation I found to be largely a matter of time. Using a 0·05-per-cent. solution of the dye, I found that the addition of even 1 per cent. of potassium chloride precipitates a considerable portion of the dye in 24 hours. Immediate precipitates such as I had obtained in the nerve were only to be obtained by the addition of much larger quantities of the salt, beginning at about 5 per cent. Such precipitates, however, no matter how rapidly formed, betrayed a crystalline structure when examined with the microscope. The rapidity of precipitation is greatly increased by the addition of dextrose to the solutions of the dye, but here again the precipitate is crystalline. It seemed possible that the granular character of the precipitate within the nerve might be the result of the viscosity of the solution present there, and this made me try precipitation experiments in strong solutions of gelatine. Even here, however, I was not successful, although there were certain suggestive alterations in the grouping of minute crystals to be observed. On the other hand, I was able to obtain spherical granular precipitates of the dye in a manner which only seemed to confuse the issue. Thus, bile-salts precipitate the dye in just the form required. So also do solutions of Witte's peptone. The addition of blood-serum to solutions of the dye resulted in the precipitation of yellow granules exactly resembling the granules seen in the nerve-fibre, when the amount of serum added was such as to ensure a precipitation of globulins. I was, therefore, placed in a new perplexity as to the meaning of the granules observed in nerve-fibre, when, fortunately for my conclusions, Macallum's paper was published. The drawings accompanying this paper at once convinced me of the fact that the differential distribution of the hypothetical salt solution deduced from the neutral-red staining did indeed represent an actual differential distribution of potassium salts. The drawings given by Macallum correspond exactly with the appearance seen by me, with the exception of some slight changes produced by the acid contained in his reagent upon the myelin sheaths. Axis-cylinder for axis-cylinder, the appearances represented by Macallum and those seen by me are the same. It is unnecessary to say that his method is far superior to mine, that will be gathered at once from the ambiguities related above. Neutral-red has, however, this advantage over the definite precipitant, that the effects produced can be watched much more readily in the course of their development.

I have obtained Macallum's reagent from the source from which he obtained it, and have used it in the manner described by him. As a result of my observations of its action I am in a position to extend his observations and give to them an entirely different significance. Not only are certain nodes of Ranvier, and various unexplainable intermediate points affected in the manner described by him, but every site of injury, and amongst these the intermediate points alluded to must be reckoned, is similarly affected. The cut ends are affected in the same way, as are also points purposely injured in the length of the fibres—as by placing a hair across a bundle of fibres and compressing a limited section of each fibre. Teasing the nerve in normal saline and leaving the teased fibres for some time in this solution before the introduction of the precipitating reagent, allowing the injury-current some period of action, the precipitate is given a new and much more extended distribution. It follows, therefore, that a new interpretation must be placed upon these results. *Since precipitates of potassium salts can be obtained at any point arbitrarily selected as the site of an injury, these salts are, therefore, really present at every point in the course of the nerve-fibre, and that too in astonishing quantity. The presence of these salts is only revealed where the axis-cylinder is involved in the process of injury.* Further, an examination of such preparations gives no support to the idea, that these are situations to which the precipitating reagent has readier access. The results are exactly comparable to those obtained with neutral-red. In the latter case there is no doubt of the fact, that the dye may be present in maximal quantity in long stretches of the fibres in which no such appearances are seen; indeed, it is frequently the case that such parts are more deeply stained than tracts in which there is a well-marked granular formation.

This revelation of a strong solution of electrolytes at every injured point, and the successful concealment of a similar quantity of inorganic salts at every other point, seems worthy of the most serious consideration. It has long been supposed that the state present at an injured point was comparable to the transitory state of excitation involving normal points of the nerve. Let it be assumed, as it has often been assumed, that it is merely a more fatal and irrecoverable degree of the same kind of change, then the observations of this state recorded above contain a sufficient explanation of the phenomena of nerve-conduction. It is better to take the pure phenomenon of nerve-conduction, the characteristic function of nerve, apart from a consideration of its outwardly recorded symptom, the action-current; for the latter cannot be considered without dealing with the permeability of the nodes of Ranvier, and the effect of this permeability upon the relative velocity of anions and kations traversing it. The picture of the process resulting from injury as observed above is as follows:—

(1) A suddenly produced change at the injured segment resulting in the local liberation of inorganic salts into a new state of simple aqueous solution. (2) A transmitted effect observed in the neighbouring portion of the axis-cylinder, probably involving an alteration of the colloidal state of the solution; for such I take to be the teaching of my attempts to obtain spherical granules instead of crystalline precipitates with neutral-red. (3) Evidence obtained from an observation of the granules, that even the injury-current is on some occasions brought to an abrupt termination by the complete disappearance of its source, the electrolytes being again suddenly replaced in the concealed position, or condition, from whence they have come; and thus the conclusion that change is therefore even in this case sometimes a reversible one.

Let us then suppose that in all these events we have a modified representation of the process of excitation and its consequences upon neighbouring segments of the fibre; a reversible change during which electrolytes are set free into a state of simple solution, and are then recovered from this state back into their original condition. Here truly there is the appearance and the withdrawal of a source of energy, a relay placed at every point of the nerve to ensure the continuous propagation of the excited state. Inorganic salts are set free to move; they move ever so little; the next segment of the fibre is charged as a consequence (let us say negatively); the colloidal state of the fibre is thus changed from its condition of equilibrium; as a result a setting free of electrolytes at this new point and the propagation of the process; in the meantime the communication of the negative charge to the onward segment has left the original segment positively charged, the state of colloidal equilibrium is thereby reproduced, and the last involved segment is brought to a condition of rest. The idea of such a progressive fall from and return to a condition of colloidal equilibrium has already been advanced in explanation of this phenomenon, the novelty is an introduction of a new source of energy with the justification of actual observation.

The manner in which I have been able to associate the collections of potassium salts observed by Macallum with the process of injury—and possibly with excitation—in the case of the nerve-fibre, must necessarily have some bearing upon the collections found by him in the other excitable tissue examined, *the muscle-fibre.* In the muscle-fibre he has depicted the presence of strong solutions of potassium salts in one only of the two sets of alternately-arranged discs of which each fibril is composed. In this case, also, attention must be drawn to the fact that here we have a picture of alternately-arranged regions of high and low osmotic pressure. Is it not possible that in this case also we have a picture, not of a resting, but of an excited tissue?

The acid character of his reagent is in itself a ground for assuming that this is the case. Let us suppose that the fibre was excited to a state of contraction by the acetic acid contained in his reagent; that the process introductory to contraction was the sudden liberation of inorganic salts into a state of simple solution; that the facts of contraction were due to the new conditions of osmotic pressure so produced. The liberated salts being immediately precipitated by his reagent, it might even happen that such a train of events could occur without any of the external manifestations of the contracted state being observed.

Again, Macallum has depicted similar strong solutions of potassium salts as present in the units of structure responsible for the flow of water in the sap of plants. He describes these solutions as the passive relict of previously transmitted solutions of potassium. Such solutions are supposed to have passed through; the water has evaporated, and cumbersome collections of salts have been left behind. Here, again, however, it must not be lost sight of that these salts were in a state of solution when precipitated by his reagent, and were therefore capable of exerting their due value of osmotic pressure. It seems possible in this case also that he was dealing with an excited tissue; that these regions of high osmotic pressure represent the cause of the transmission of water, and not its after consequences.

REFERENCES.

1. J. S. Macdonald, 'Thompson Yates Laboratory Reports,' vol. 4, Part II, pp. 213—348, 1902.
2. ,, 'Proc. Roy. Soc.,' vol. 67, pp. 315—324.
3. 'Proc. Roy. Soc.,' vol. 67, pp. 325—328.
5. ,, 'Proc. Physiol. Soc.,' December 17, 1904.
6. ,, 'Proc. Physiol. Soc.,' March 18, 1905.
7. A. B. Macallum, 'Journal of Physiology,' vol. 32, p. 1.

ADDENDUM—Received May 13, 1905.

Summarising in a former paper (1) all the evidence then obtained as to the distribution of inorganic salts in nerve-fibres, I made use of a statement from which perhaps a quotation might now be permitted. Since, for purposes of criticism, it clearly defines the personal point of view from which this present investigation was undertaken. "Accepting all that is taken as known of the minute microscopical structure of nerve, there is no inherent improbability in the supposition that the inorganic salts of the nerve might be there held enchained in a highly concentrated solution free to move parallel, but not at right angles to and away from the fibrillæ. Granting such a possibility, we are however faced by the important corollary that such concentrations are indeed enchained there, and are thus unable to exert an osmotic pressure, or by diffusing away, give rise to electrical phenomena. To explain, in the presence of such an hypothesis, diffusion processes consequent upon injury, it seems necessary to invent a phenomenon really secondary to injury and involving new conditions of the fibrillar structure. The invention of such a phenomenon is as culpable as the invention of a chemical change, and the necessity for doing so is equivalent to the necessity of abandoning this supposition."

Obviously the main difference between the state of the evidence then and now is, that a distinction of the kind suggested between the condition of the inorganic salts at the "cut end" and their condition at remaining portions of the nerve-fibre has been actually observed. It follows, therefore, that the original hypothesis, temporarily rendered absurd by the necessity for such an assumption, is now remarkably strengthened by the conversion of the assumption into a fact.

There is, however, another difference of importance, which may well be dealt with at once before proceeding to a consideration of the main point. In doing so it may be necessary to anticipate conclusions arrived at more logically at a later stage. This difference is that, in the statement quoted above, the fibrillæ are mentioned as veritable units of structure of the nerve-fibre; whereas in the preceding part of this present communication they have been neglected, and the axis-cylinder has been dealt with as if it were a homogeneous colloid solution. This change of opinion is considered justified by the following facts. My personal acquaintance with the microscopical appearances of nerve-fibres has since then largely increased. Whilst cultivating it in pursuit of the present histological investigation I have used dyes suitable for a revelation of neuro-fibrils. I have, however, never seen these neuro-fibrils, except under such circumstances as demanded their criticism as possible artefacts, as for example after the use of fixatives, or in short stretches of nerve-fibres obviously suffering from the results of excitation. Yet the only peculiarity marking the use of these dyes in this investigation has been the fact, that they have been for the most part presented to the fibres in solutions of such salts as were least likely—as previous observations had shown—to destroy the "living state" of the fibres.

In place of these neuro-fibrils, however, the granules spoken of have made an absolutely constant appearance. Such granules I have observed forming at the cut ends of the fibres, but also in portions of the fibres remote from positions of injury. Such granules may, even in portions of intact fibres, be observed to increase and again to diminish in size. They may be seen at any one time to be of different sizes and in different number in neighbouring portions of the axis-cylinder. There is, therefore, no temptation to consider them as permanent units of structure, and yet, after the admission of "fixatives," these very granules are joined together in lines to form neuro-fibrils. The granules not being permanent units of structure, it is therefore logical to conclude that the fibrils thus formed by their agglutination also are not permanent units of structure. The fibrils then may be said to be observed under conditions suggestive of artificial formation. This fact also has to be taken with the complementary fact, that artefacts of just this kind might be expected to appear within the nerve-fibre.

Let us in illustration of this statement consider the possibility mainly dealt with in this paper, that the process of coagulation is attended with a sudden liberation of inorganic molecules, and therefore with a new condition of activity—molecular motion—around every centre of coagulation. It seems not unlikely that, in the extremely minute tubule of the nerve-fibre, this new activity, and the conditions of pressure occasioned by it, might systematise the spatial arrangement of these centres of activity along lines parallel to the long axis of the fibre. This being the case, there seems an ample basis for the opinion, that we should expect a symmetrical arrangement of lines of coagulated material. In fact given the sudden appearance of a uniformly distributed agency determining coagulation, we should expect the formation of "neuro-fibrils" as a consequence of the energy changes accompanying this process. I shall continue then, in this paper, to speak of the axis-cylinder as a homogeneous colloid solution. This point defined, it is possible to turn more freely to a consideration of the disturbance of this homogeneity taking place at each site of injury.

Evidence that the appearance of potassium salt at a site of injury is due to physical and not to chemical change can partly be found in a consideration of the quantity of the change observed. I have found it impossible to secure a sudden precipitation of neutral-red, one of the dyes used for the detection of this change, by the addition of potassium salts, unless the salt is added in very considerable quantity. A small quantity of the salt, 0·1 per cent., will determine a considerable precipitation in a considerable time. The precipitation occurring in the nerve is however sudden, and can only be imitated by the use of concentrations of more than 5 per cent. The same point was brought out by my examination of the amount of potassium salt required to annul the injury-current. If it is admitted that the quantity is considerable, then it follows that if its appearance were really due to a chemical change, we should expect an appearance of other products of this change in similar considerable quantity. And yet the ingenuity of observers has been exhausted in the endeavour to discover almost every conceivable kind of product of change other than this one, which has now been discovered. The acid products expected from a consideration of the apparently analogous case of muscle-injury have been shown in the course of this research to be definitely not present. There is no longer any reason to consider their existence as probable, since the fact has now been recorded that the site of injury may be neutral, and even alkaline, at a time when the process of injury is most effective in the production of its consequence, the injury-current. So far, therefore, there is no reason to consider the existence of any process of chemical change.

Again, when attention is paid to the extraordinary series of exciting causes, which can be used to provoke this change, further doubt must be thrown upon the suggestion that it is the result of a definite chemical reaction. The similarity in consequence of the application of slight modifications of temperature in both directions of the scale, of slight mechanical interference, of acids and alkalies, of other chemical reagents having apparently no common basis of chemical action, clearly indicates the improbable nature of the statement that the energy thus disclosed has been previously locked up in some form of chemical combination. The indications are therefore such as to demand a search for some physical bond between these salts and the remaining constituents of the axis-cylinder removable by a great variety of circumstances.

Our attention once directed to the physical conditions present within the axis-cylinder, it is seen that the most remarkable condition present is the yet imperfectly understood condition of proteid material in a state of colloid solution. The facts of the case, therefore, so far as they have been elicited, may be accepted as an indication that this state of colloid solution is in some way a possible store of potential energy. If this is so, then the state of more perfect colloid solution should rationally be expected to function as a more ample store than any state of less perfect solution. It therefore becomes a matter of importance to inquire whether any diminution in the perfection of the colloid solution of the axis-cylinder, any tendency towards coagulation, has been observed in the region of the nerve-fibre in which this redistribution of energy has been found.

It is at this point that my observations of the staining effects of neutral-red and toluidin-blue come to the assistance of those made with the aid of Macallum's reagent. They may be accepted as evidence of the deposition of colloid material (gel.) as granules of visible dimensions, upon the surfaces of which the dyes are precipitated from the solutions of inorganic salts surrounding them. In medullated nerve therefore a colloid solution is found, the condition of which is modified by injury and presumbably by excitation. When a more perfect state of colloid solution is degraded to a less perfect state, this fact is attended by a rise in osmotic pressure due to the liberation of electrolytes into a new condition of simple aqueous solution. The existence of a colloid solution with similar qualities in non-medullated nerve is inferred from the bulbar prominences, which at every site of injury form evidence of the effects of an increase in local osmotic pressure. Similar bulbar prominences occurring under similar conditions upon naked nerve-endings, and upon the dendrites of nerve-cells, point to the universal distribution of such a solution throughout the whole scheme of nervous tissue.

In explanation of this rise in osmotic pressure accompanying the observed degradation of the state of colloid solution, I will take it as possible that, in the colloid solutions here considered, a very considerable fraction of the mass of inorganic salts concomitantly present is situated upon the surfaces of the aggregates of colloid molecules. When, therefore, the total surface presented by such aggregates is increased by their more minute state of subdivision in the state of more perfect solution, the relative quantity of electrolytes locked up upon such surfaces may conceivably be increased, and the quantity in a state of simple aqueous solution diminished. On the other hand any desolution of the colloid may result in an increase of the quantity of electrolytes present in aqueous solution, and in a rise in osmotic pressure.

Many instances are known of the collection of such a condensed solution of salts upon the surface of solid colloid masses, such as the surfaces of threads lying immersed in aqueous solutions of salts. It seems feasible that the conditions essential to their collection are also present when the solid masses of colloid material are infinitely reduced in size. The ocular demonstration of such a condition upon particles of visible size should not be difficult, and, indeed, may be obtained from an examination of Macallum's drawings of the potassium ring surrounding granules within the nerve-fibre itself. It is possible, also, that Macallum's observations of the collections of potassium upon the surfaces of certain tissue-cells may afford a demonstration of this condition present upon colloid aggregates of a larger size; and that the Golgi staining method, and all similar impregnation methods, may depend upon similar effects.

Such an hypothesis carries with it the corollary, that the limited motion permissible to these electrolytes must diminish their usefulness in the transmission of an electrical current through the colloid solution. This corollary, therefore, opens up an objection to the hypothesis. The hypothesis would seem to be rendered unlikely by observations of the electrical conductivity of colloid solutions containing electrolytes, as, for instance, the conductivity of lymph, and, indeed, of nerve itself. Before this objection can be dealt with, another corollary must, however, be considered. Viewed from this hypothesis, colloid solutions must be considered as in a peculiar condition, which we may call "pseudo-polarisation," when placed at a different potential in different parts of their continuity—as during the transmission of an electrical current. *A colloid solution placed as a conductor in the path of an electrical current should possess a graduated series of states of perfection distributed throughout its length.* The extreme case of degradation would be found at one end of the solution. At this point, therefore, a large quantity of electrolytes would enter the state of simple aqueous solution, thus diminishing the resistance. Diminution of resistance at this point would, however, alter the distribution of potential, and the neighbouring portion of the solution would in its turn enter the same, or nearly the same, extreme condition of degradation. The resistance of the solution would in this way become increased at one electrode, diminished in the neighbourhood of the other; and in only one point, which from analogy to the case of nerve (probably very exact analogy) might be called "the indifferent point," would the colloid solution retain its original state of perfection. Thus the measurement of electrical conductivity, even by rapidly alternating currents, might provide a very false estimate of the distribution of electrolytes within a colloid solution in its original condition of homogeneous equilibrium. The hypothesis, therefore, is not at once rendered impossible by these measurements.

The possibility of such a "pseudo-polarisation" is not drawn from the imagination. Examining under the microscope nerve-fibres staining with neutral-red and toluidin-blue, I have observed regions of the axis-cylinder affected in just this way. Such regions, which are not those directly affected by diffusion inwards from the source of the injury-current, but are more distant regions, carrying the current arising from the injury-current source, are beset with stained granules, arranged very regularly within the axis-cylinder, varying in number and in size in a very definite way. Wherever the number of the granules counted across the axis-cylinder is large, the size of each individual granule is small; wherever the number is small, the size is great. Further, the number, and therefore the size, varies in a very definite manner along the region affected. So struck was I at first with the possible relation between the appearances observed and the passage of an electrical current, and so affected by the observation that these appearances occurred in regions of the fibre not directly involved by the injury, that I associated them with the possible transit of an action-current; now, however, I should rather regard them as polarised regions of the fibre involved secondarily in the transmission of the injury-current. In this connection I might put forward the claim that *the observation of an axis-cylinder carrying this injury-current provided the very best means for examining the state of a colloidal solution traversed by absolutely parallel stream-lines of current.*

In direct support of the possible existence of such a condition of "pseudo-polarisation," I might also mention the "true longitudinal polarisation" of nerve. Place a piece of nerve as truly in a straight line as possible between non-polarisable electrodes, and pass a current through it, then, on removal of this current, a "polarisation" (?) current is observed to traverse it in the reverse direction. This phenomenon has attracted some attention, since the whole of the polarisation phenomena of nerve have been attributed to the passage of the current through the walls surrounding the axis-cylinders, and in this case it is not obvious that any such traverse has taken place. It is true that an explanation has been found for it in the assumption that the fibres have not been placed in true rectilinear fashion between the electrodes. To me, however, this explanation has never appealed, possessing considerable faith in the relatively greater magnitude of the conductivity of the axis-cylinder, and not aware that slight twists in the direction of an insulated wire produced much leakage of the current through the insulation. I have also, however, observed the phenomenon under conditions in which such twisting could not be supposed to have occurred to any degree worth mentioning, as, for example, when thin slices of a stout nerve-trunk were placed in this manner between the electrodes.

Imbued with the idea that "membranes" formed a necessary factor in the production of all the internal polarisation phenomena occurring in living tissues, I was at the time inclined to postulate the existence of theoretical membranes arranged transversely to the longitudinal axis of the nerve-fibre, perhaps at the nodes of Ranvier. During the present investigation, however, I have frequently watched the granular conditions I have described pass uninterruptedly through the nodes, and have thus become convinced of the uninterrupted continuity of the axis-cylinder, and, therefore, of the absence of transverse membranes from these nodal points. This newly-described condition of "pseudo-polarisation," however, provides a complete explanation of the phenomenon of true longitudinal polarisation, just as it at the same time provides an explanation of the manner in which the electrolytes of the nerve may be set in motion in measurements of electrical conductivity, although usually locked up in its normal state of colloid solution.

The condition of "pseudo-polarisation," therefore, has certain direct evidence in support of its existence, and, further than that, its existence can be considered as an explanation of a hitherto unexplained condition. Its existence diminishes the value which might otherwise be placed upon the measurements of the electrical conductivity of colloid solutions. They cannot be used as objections to the assumed method of arrangement of inorganic salts in the colloid solution of the nerve-fibre, in which there is good reason to suppose that "pseudo-polarisation" is an easily elicited phenomenon. The hypothesis may therefore meet with serious consideration, and with it the conception that such colloid solutions as exist in the nerve-fibre form ample stores of energy in the manner suggested.

There are of necessity limits to the part which it is suggested these stores of potential energy play in the redistributions of energy observed in living tissues. In the first place, it is not suggested that the extreme amount of energy accumulated by them is in every case expended. Hitherto, in the case of nerve injury, we have been considering the extreme case, the generally fatal accident, in which the primary conditions essential to storage have been suddenly and completely annihilated. It is reasonable to suppose that such extreme instances of coagulation are not of normal occurrence, and that, within the living organism, departures from the normal state are only permitted within readily reversible limits.

Let us consider the case of a simple cellular unit, one of the many other similar units placed side by side in the formation of a tissue. Let us take the case of a single ciliated epithelial cell. Within the cell itself are certain surfaces limiting the movements of salts, but permitting a movement of water. On one side of one of these surfaces inorganic salts are suddenly liberated, there is a translation of water across the surface, the cilia are rendered turgid and are moved. Further, the movement of molecules of inorganic salts, although limited, is not entirely prevented; there is a tendency for diffusion to take place from this cell into its neighbour, the two cells are thereby oppositely charged; the electrical charge bestowed upon the neighbouring cell excites this in its turn; the charge left in the original cell brings its colloid solution back into, or nearly into, their normal state. The cell, therefore, is excited, performs its function, excites its neighbour, and itself returns to rest. In this we have an essential unit of a picture of ciliary motion traversing a sheet of epithelium in a definite direction. We have not, however, the whole of the essential unit. In the performance of this cycle of change external work has been performed, the store of potential energy has therefore been necessarily reduced. To succeed absolutely in returning to its original state, the cell must necessarily resort to a transaction with some external source of energy. True, the amount required may not be as great as at first sight appears, since there has been a give and take between neighbouring cells. Minute as it may be, however, there can be no doubt as to the loss of energy from each individual cell.

It is impossible, therefore, to accept the physical theory dealt with here as accounting for all of the phenomena observed. That does not seem to me, however, a fact detracting in any way from the significance of the statements made above. The "head of steam" immediately made use of in the contraction process may, nevertheless, be sought in the sudden removal of conditions hitherto restraining the motion of inorganic salts. In the case of the transmission process from cell to cell, we may greatly strengthen the form of the statement. The condition essential to this transmission must almost necessarily be of the kind considered. It is conceivable, therefore, that the most interesting elements of the function of the cell might be more economically studied when attention is mainly directed to the alterations in physical condition occurring within and around it, than by a direction of attention to the ground-phenomena of chemical assimilation and dissimilation.

In the case of nerve-conduction, there is least need to place the chemical changes of assimilation and dissimilation in the fore-front of an explanation. Such changes are undoubtedly facts, but it has still to be proved that they are facts requiring primary consideration in connection with characteristics of function. In the case of nerve, the amount of external work done is infinitesimal. The tissue's primary duty is the transmission of energy, and it accomplishes the purpose of its existence with remarkably little loss. The nervous impulse also is transmitted with a rapidity, and with an absence of trail, not usually observed in the transmission of chemical change. If the nervous impulse is a chemical phenomenon, then it is a most marvellous chemical phenomenon. If it is a physical phenomenon, it falls at once into a general class of physical phenomena, also leading to the transmission of energy from point to point. As a physical phenomenon its nature may not be understood, but it is easy to conceive that it soon may be understood, and that it will prove to be a simple phenomenon. If it is a chemical phenomenon, then it is almost necessary to conceive that it is a complex process, obtainable also, only under the most complex conditions. There can be no objection, therefore, to the introduction of another physical theory of nerve-function, if supported by sufficient evidence to warrant its introduction. The more simple the theory, the more likely it is to be true.

Let us therefore think of an isolated strip of colloid solution containing, and capable of containing without suffering precipitation, a large quantity of inorganic salts. Let us also grant the existence of a peculiar relation between colloid material and inorganic salts of the nature previously described.

Apparently the only other conditions necessary to imitate nerve-function, and they at least are entirely reasonable conditions, are that the colloid solution should undergo some degree of a coagulative change when negatively charged, and that the negative anion of the contained inorganic salts should be relatively more rapid in its motion than the positive kation.

Let us think of the strip as A, B, C, D, E. At A, the terminal point, let any agent be applied productive of a condition of degradation (tendency towards coagulation). The condition of degradation is at once the cause of a sudden rise in osmotic pressure due to the new freedom given to the inorganic salts. Instantaneously diffusion tends to take place into B, and the instantaneous result will be the conference of a negative charge upon this neighbouring portion, a positive charge upon the portion first affected. Point B is therefore now excited, and point A, originally excited, is brought back into a state of rest, and indeed, by reason of its positive charge, beyond that state into a condition of more perfect colloid solution than before. A has passed from a condition of excitation into a condition of inhibition, a condition however which it does not long retain; since backward diffusion from the newly excited point B reduces its positive charge, and brings it again to its original normal state. Point B at first made negative and thus excited is the seat of an increased osmotic pressure, and thus electrolytes are tending to move from it both backwards towards A, thereby annulling the temporarily positively charged state of A, and forwards to C, thereby conferring a negative charge upon C and producing in it the state of desolution and excitation. Each point in succession—point B may be taken as a typical instance—is first made negative, then positive, then returns to its state of rest.

Considering the process in terms of the state of electrical potential, the forward movement of the negative charge is ensured, or rather its backward transmission is prevented, by the pursuing positive charge. Considered in terms of osmotic pressure the facts seem somewhat simpler, since it may or must be considered that the rise and fall of osmotic pressure respectively lag a definite time behind the appearance of the causes producing them. It is also conceivable that the rise, the release, is a more sudden phenomenon than the fall, the recall. The facts then arrange themselves in this manner. The rise of pressure in B occurs at a time when there is still a region of increased pressure behind it at A, but a region of normal low pressure in front of it at C. The tendency is therefore always forwards. Arranged in these terms the wave of the nervous impulse can be described as a double oscillation in the value of osmotic pressure, the front a rise, the trough a fall.

In order to connect what has been already said of the nature of the "pseudo-polarisation" of nerve, and this scheme of nerve-function, it may be well to consider briefly the relation existing between some of the physical and functional changes produced by the action of a polarising current. "Pseudo-polarisation," unlike true polarisation, is fully developed within a very short time from the institution of the current. The time required being that taken by the colloid solution in responding to a difference of potential. At the kathode desolution has occurred, and the corresponding rise in local osmotic pressure. At the anode the contrary change has taken place, and the osmotic pressure has fallen. At some point intermediate between the poles the normal state is maintained. At the moment of current closure, the osmotic pressure at the kathode is relatively much greater than the osmotic pressure at a point immediately beyond the kathode than it will ever be again during the continuance of the current, the difference being due to a distinction between the conditions present at the moment of establishment of diffusion, and those present after a steady condition of diffusion has begun. The negative ion being the faster, this is the only moment when the point beyond the kathode is negative to the kathode itself. At this moment, therefore, this negativity, due primarily to the initiation of diffusion, produces an excitation in the extrapolar region, and thus causes the travelling nervous impulse characteristic of current closure.

In an extremely excitable nerve it might be expected that the wave of positivity, and therefore of lowered osmotic pressure following in the wake of this transmitted process, might provide an opportunity for a new and almost equally sudden diffusion from the kathode, and therefore for a repetition of the travelling excitation. In such a case a series of excitations might be started from the kathode, and give rise to a tetanic response of the motor organ. There is no difficulty in the way of providing a complete explanation for the kathodal phenomena occurring at the moment of current closure. The ease with which this can be done, however, entails some discussion as to the reasons which determine the absence of a transmitted positive "inhibitory state" starting from the anode, travelling along the nerve with a wake of negativity behind it. If the fall of osmotic pressure at the anode was as great as the rise of pressure at the kathode, then the use of the same hypothesis would indicate the necessity for such an occurrence. The fall of osmotic pressure at the anode is, however, in fact, less than the rise at the kathode, and this is determined by the following consideration.

In the state of normal equilibrium of the colloid solution, the amount of inorganic salt present, in a state in which it can exert an osmotic pressure, is comparatively small. The concentration of the salt solution at this time is about that of a decinormal solution. When complete coagulation of the colloid has taken place, there is reason for believing that the concentration is very much greater, being somewhere about ten times as great. A fall of pressure below the normal never involves any great relative difference of pressure, a rise of pressure, however, may entail comparatively great relative differences. This distinction would seem sufficient to explain the comparative insignificance, and even the complete absence, of the transmission of an "inhibitory nervous impulse" starting from the anode.

When considering the functional changes observed during the time of closure of the current, it is necessary to reckon two sets of physical conditions. The one set, determined by true polarisation, taking place on the surfaces of the partially permeable sheath of the nerve-fibre, that is, at the nodes of Ranvier. The other set, determined by "pseudo-polarisation." There is a certain similarity between the effects of these two conditions occurring at each pole. Their consequences are added together in the production of the anelectrotonic and katelectrotonic arrangements of differences of potential. The two factors, however, have different time relations, the effects of true polarisation gradually increasing, the consequences of "pseudo-polarisation" being most marked to begin with, and subsequently declining. The polar conditions due to true polarisation are sufficiently well understood. Here it is only necessary to dwell upon the polar conditions due to "pseudo-polarisation."

During the early stages of current closure, the condition of "pseudo-polarisation" gives rise to an increased osmotic pressure in the region of the kathode, a diminished osmotic pressure in the region of the anode. The partially permeable membrane of the nerve-sheath is more permeable to the negative anion than to the positive kation. An increase in osmotic pressure will therefore lead to the separation of diffusing ions in the line of this sheath. The diffusion is in this case outwards, and the faster negative ion therefore communicates its negative charge to the parts outside this sheath, that is to say, to the surface of the nerve. Each point in the kathodal extrapolar region nearer to the kathode is therefore more negative than more distal points. The condition due to true polarisation is therefore reinforced. At the anode, the region initially of diminished osmotic pressure, diffusion is inwards, and the external surface of the nerve possesses the charge of the more slowly diffusing positive kation. Here, then, the condition of anelectrotonus, due to true polarisation, is reinforced. From reasons given above, the addition at the anode is much smaller than the addition at the kathode. The anelectrotonic condition is therefore at first smaller in magnitude than the katelectrotonic condition, both also at first increase in magnitude with the increase of true polarisation. The condition due to "pseudo-polarisation" is, however, undergoing a diminution with lapse of time, possibly at once accounting for the subsequent serious fall in the value of katelectrotonic condition. To explain this, it is necessary to spend a few moments with the details of the condition of "pseudo-polarisation."

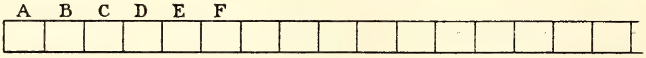
The essence of the condition of "pseudo-polarisation" is the arrangement of the colloid particles of the axis-cylinder in a series of graduated sizes between the poles. The particles (aggregates of molecules) at the anode are smaller than those occurring in normal stretches of the nerve, those at the kathode are bigger and less numerous. The immediate consequence of this arrangement is the motion of inorganic salts in large quantity in the kathodal region, in small quantity at the anodal region. This, however, is a condition which cannot remain as permanently as the cause of its original appearance. The salts diffuse, and finally their distribution must, at least, become far more uniform, and that, too, before the essential condition of "pseudo-polarisation" has been removed. The electrical consequences of the differential distribution of osmotic pressure inaugurated by "pseudo-polarisation" are therefore evanescent.

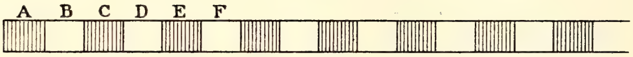
The functional changes of excitability at the poles are primarily dependent upon this complicated addition of states, and to them there has still to be added the chemical consequences attending upon true polarisation. Where there are so many factors to play with, there can be no difficulty in explaining any modification in excitability which may arise. It is doubtful if there is any advantage gained by doing so. For the present, it may only be pointed out that there is ample reason to expect a marked difference between the earlier and later stages of these alterations in function.

Having considered in this preliminary way some of the classical phenomena produced by the passage of a current through nerve, it is possible to return to the more interesting subject of function. We see that the nerve owes its function to the possession of this core of colloid material, and to the property of "pseudo-polarisation," which is an attribute of the colloid material. There is still, however, another point to expand. Stress has been laid above on the apparently necessary condition, that the more rapidly moving ion of the electrolyte of the nerve should carry an electrical charge of the same sign as that which leads to the desolution (tends to lead to the coagulation) of the proteid colloids of the nerve. Introduction of the negative ion being the condition leading to desolution of the colloid and liberation of the electrolyte, then, if the process is to be transmitted, the motion of the liberated electrolyte must result in the bestowal of a similar charge upon the neighbouring portion of the colloid solution, and therefore the negative anion must have the greater relative velocity.

Let us consider what would happen if this were not the case. Let us deal with an assumed case, in which the bestowal of a negative charge being the condition essential to desolution, the positive kation has the greater relative velocity. Let ABCDE represent such a strip of colloid material. To begin with, the strip is homogeneous, and the distribution of potential, and of osmotic pressure along it, is uniform. Now, from any exciting cause whatever, let us suppose that the solution in A undergoes a change that in it desolution takes place, and electrolytes are set free. Diffusion tends to take place, therefore—the positive ion having the greater velocity—B becomes positively charged, and A is left negatively charged. The condition originally excited in A is hereby exaggerated, and more electrolytes are liberated, provided there is still a further stock to call upon.

This fact would seem to indicate that there will be no further progression of such a process along the strip ABCDE, seeing that it certainly determines a more prolonged maintenance of the phenomenon in each spot affected. This, however, is not necessarily the case. B has become positively charged, its colloid particles have therefore gone into a state of more minute subdivision, and some of its inorganic salts have been consequently removed from the state of simple aqueous solution. The osmotic pressure in B has fallen. Considering the effect of this upon C only, we perceive that a diffusion will now tend to take place from C to B. Thus, C will now become negative, and, consequently, the site of liberation of electrolytes, and of increased osmotic pressure. Similarly, in its turn, D will become and remain positive, E negative. Further onwards, every alternate segment will similarly be rendered negative, and the site of an increase in osmotic pressure, until the process has dwindled away, as finally it necessarily must.





The process is therefore, within limits, transmissible, and, although evidently unsuited as the basis of function of a tissue, the prime necessity of which is conduction, is, nevertheless, possibly suited to the requirements of a tissue in which the maintenance of a state of increased osmotic pressure is required in alternate segments of the tissue. Conceivably, such a process might be suited to fulfil some of the requirements of striated muscle. Glancing at the accompanying diagram, we see the result of such a process in an arrangement of alternating segments of high and low osmotic pressure respectively. Compare such a diagram with Macallum's representation of the potassium salts in muscle, and there is a similarity which is at once worthy of examination.

I have already stated above that, in my opinion, Macallum's representation must be interpreted as evidence of the existence in muscle (probably in the contracted state) of such a disposition of alternating levels of pressure. Nor need we depend only upon this observation, since Schäfer has, some considerable time ago, recorded observations depicting a flow of water from disc to disc of the muscle sarcostyle, such as this condition of alternating segments of high and low osmotic pressure would determine. There is therefore ample reason to inquire further into the possibilities of this process.

Let us for example inquire how such a strip of material would react to a mechanical extension. Mechanical stimulation it is safe to assume would lead to a general tendency towards desolution along the strip in each of the segments into which we have divided it This tendency, from reasons previously given, would affect most the segments in which desolution had already taken place. In other words, an increased load would lead to an exaggeration of the preceding condition of contraction.

Let us turn to the contracted states of muscle, in which it is known that coagulation has taken place—to "rigor mortis" and to "heat rigor." Apply a load to muscle in either of these two conditions, and the contracted state is not increased, and why? Obviously because (1) the maximum coagulation has already taken place, and (2) because the membranes dividing the muscle-fibril and also the muscle-fibre into actual segmented parts have been injured. Remove these segmenting membranes and there can be no further contraction, since alternately arranged foci of high and low pressure can produce no externally felt alteration in the whole bulk of the muscle unless definitely separated from one another by partitions preserving their distribution.

In muscle, therefore, we must raise to a high functional value the transecting membranes, which have no place in the function or structure of nerve except that of insulating each individual nerve-fibre. We must, therefore, when we come to study the effects produced in muscle by the transmission of an electrical current, expect to find the relative importance of true polarisation and "pseudo-polarisation" changing places with one another. We must therefore also expect to find, in the case of muscle but not of nerve, an appearance of new chemical substances originating from the processes of hydrolysis taking place at veritable "poles" situated at frequent points within the continuity of the muscle-fibril. In fact in muscle, these processes of polarisation and hydrolysis are probably the servants of another useful purpose. The osmotic changes in nerve were self-annulling, the whole process admitted of a purely physical explanation. In muscle, as I have depicted it, the process is different in this particular. The conditions evoked by it are such as to ensure their own permanence.

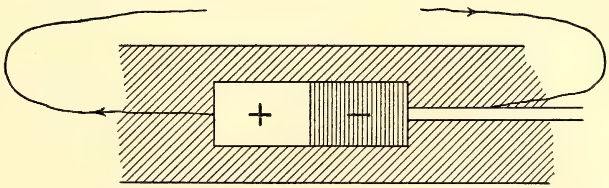
It is obvious that the difference must be so. Nerve is a material adapted for the transmission of energy from point to point. The external work done in nerve-conduction is infinitesimal. In muscle, the performance of external work is the main duty of the tissue. A transmitted excitation proceeding along a muscle-fibril with an inhibition in its wake would be valueless, or of value only as secondarily exciting to some other consequence. Such a phenomenon might be supposed to occur in the sarcoplasm, provided that a phenomenon of another kind took place in the sarcostyles.

This being the case, it is necessary to provide some factor other than this which shall secure the relaxation of a muscle following upon a sustained contraction. Conceivably this factor is to be found in the internal true polarisation taking place in every segment of the muscle-fibril; the separation of alkali at one pole and acid at another. The further removal of acid out of the muscle may also be conceived to serve some functional end. Thus in our theoretical strip with its alternate segments of high and low states of colloid solution, segments which we have now decided must be definitely divided off from one another by intersecting membranes, let us suppose that alkali is formed in the segments where the colloid particles are now larger than normal, that acid is formed in the segments where the state of more minute subdivision exists—and this as a secondary consequence of the existence of these states and of the changes in osmotic pressure so caused. It is conceivable that these chemical facts might be prominently useful in the return of the solutions, the one up to, the other down to its resting state. In other words, whereas contraction is probably a physical fact produced primarily by physical agency, relaxation must be (see above) to some extent a chemical fact and may be initiated by the chemical processes resulting from polarisation.

Assuming this hypothesis to be correct, and muscular contraction dependent upon the excitation of a "pseudo-polarisable" colloid solution containing an electrolyte of the kind supposed, it is obvious that a strip of homogeneous colloid solution might become segmented in the manner of the muscle-fibril as a consequence of its initial participation in function. Excite such a strip a small number of times in comparatively rapid succession. Arouse in it repeatedly this segmented arrangement of states of colloid solution, and it is to be expected that a permanent differentiation of this kind must ensue. The one segment is repeatedly affected in one direction, and brought back from that condition to a state of rest; its neighbour is repeatedly affected in the opposite direction and from thence returned to the same state of rest. This in fact seems so completely probable a suggestion, that one is inclined to lay stress upon it as a marked instance of the determination of structure by function.

Considering the possibilities of the manner in which the function of the muscle might be served by this creation of conditions of high and low osmotic pressure in such alternate segments, the following points seem worth noticing. In the first place there is the resulting translation of water, to which it has not been found necessary to attract attention in the case of nerve-function, because of the absence of transverse membranes. The pressure in the dim bands being raised, and that in the bright bands lowered, water will pass into the dim bands from the neighbouring bright bands. If the lateral walls of the dim bands yield to the pressure ever so little, then these bands will assume the elliptical form, which they have been seen to adopt. The result of this process therefore is a movement of water from the longitudinal axis of the fibril, and thus from the longitudinal axis of the fibre, into a series of transverse axes. This is in itself sufficient to account for the shortening and simultaneous thickening of the fibre as a whole.

It is natural to turn to the electrical fishes for a further test of the possibilities dealt with above, and it is not surprising to find in them convincing support. Let us imagine two consecutive muscle-segments, a dim band and a bright band arranged together in a stout connective-tissue covering. Let us suppose that no intervening membrane has formed between the two segments, so that no considerable redistribution of the water in the two segments (of any consequence) can result from a localised liberation of electrolytes into solution. Further, suppose that a branch of the motor nerve terminates in the dim band, so that the transmitted negative charge of the nervous impulse is bestowed upon the dim band. The colloid in the dim band becoming negatively charged, passes into the state of desolution (tendency towards coagulation), the local osmotic pressure will rise, and electrolytes will diffuse into the bright band. Now, if in this arrangement we have also retained the condition present in muscle, namely, that the positive kation of this diffusing electrolyte is of a greater relative velocity than the negative anion, then the positive charge will be bestowed upon the bright band, and the negativity of the dim band caused by the nerve discharge will be reinforced. Thus, a current will traverse this piece of tissue in the direction of the greater relative motion of the positive charge —from dim band to bright band. Surrounding conductors will be traversed by a current in the opposite direction, a current which may be described as passing in the direction of the nerved end of this little organ. This, then, is



an electrical organ, the discharge in which takes the direction defined by Pacini's rule. It is just such an organ as, from what has been said previously, we would expect to find as the result of the transmutation of a muscle into an electrical organ.

An organ, however, developed on similar lines from some other colloid solution than that found in muscle might be conceived to contain some other electrolyte, and, indeed, one in which the negative anion possessed the greater relative velocity. In this case, the segment of the colloid in the immediate vicinity of the nerve-ending would be positively charged as a result of desolution, and the more distal segment would be negatively charged by the more rapidly moving negative anion. The discharge, then, through such an organ would take place towards the nerved end of the organ, and, in the surrounding conductors, a current would pass in the reverse direction, that is to say, away from the nerved end of the organ. The colloid solution to make an organ of this new kind might conveniently be taken either from nervous tissue itself, in which the electrolyte is of this character, or it might be taken from epithelial masses elsewhere, since we may conclude, from the constant " ingoing " direction of the current detected in such masses, that here, also, the negative kation is that possessing the more rapid motion. It is not surprising, therefore, to find that the observations made upon the electrical organ of Malapterurus have served to destroy the all-sufficiency of Pacini's rule.

It is obvious, also, that once the admissibility of the conceptions thus used to explain the main facts of nerve and muscle function, and the function of the electrical organs of fishes has gained acceptance, the same conception can also be at once advanced in explanation of a large number of the phenomena exhibited by every excitable tissue. Inhibition and excitation in the central nervous system, in the heart and vascular tissues; the condition of tone; the flow of water through secretory surfaces; in fact, all conditions reacting to nerve discharges with comparative rapidity. They are also applicable to the case of unnerved tissues, such, for example, as the instance of the ciliated epithelium mentioned previously. An examination of the " blaze currents," elicited from so many tissues by Waller, might conceivably reveal many instances which might find their explanation in the use of these conceptions.

In general the conception might be stated briefly thus. A unit in a state of inhibition is one in which the state of colloid solution is more perfect than normal. In this condition the inorganic salts, and therefore the electrolytes, are mostly bereft of all the influence they possess in virtue of the number and motion of their molecules. A unit in a state of excitation is one, on the other hand, in which these molecules are in a state of motion, a condition produced by the degradation of the colloid solution of the unit. A unit in a state of tone is in a condition intermediate between these states. Again, characteristic differences between the functions of different units may in some cases find their explanation in the comparative freedom allowed to the released molecules, and also to physical differences between the molecules released, such as differences in electrical charge and rate of motion.

Returning for a moment to the special case of nerve, it might be pointed out that the hypothesis would lead one to expect the possibility of finding nerve-fibres capable of communicating a positive charge, in place of the more usual negative charge, to the tissues innervated by them. This might conceivably be accomplished in two different ways. In the one case a nerve-fibre excited itself by a negative charge, and transmitting a negative charge to successive portions of its length, might possess a small terminal portion, a membrane, which reversed the condition prevalent in the remainder of the fibre by permitting the more rapid migration of the positive ion, and checking the movement of the naturally more rapid negative ion. In this case there would be a condition as a corollary to this. The terminal portion of the fibre abutting upon the membrane would be left in a state of continuous excitation involving a prolonged communication of positive charges to the innervated tissue, and a prevalence in it of a condition of inhibition.

The other case conceivable is one in which the colloid solution of the nerve-fibre was excited by a positive charge, and contained an electrolyte the positive ion of which was the more rapid in its migration. Such a nerve-fibre would be excited at the anode of a polarising current, in the manner in which there is some evidence that certain afferent fibres are excited. It should be pointed out that, in such a case, there would be no reason to expect the "action current" to differ in kind from the action current of an ordinary nerve-fibre, the direction of the action current being primarily determined by the impermeability of the sheath of the fibre to the positive ion. The negative variation of the injury current, and the injury current itself, might differ, notwithstanding the predominant influence of this sheath, in their production also, but the degree to which this difference might rise could not be anticipated without at first exactly determining the nature of the permeability of the sheath.

In conclusion, I would like to offer a few words in explanation of the attitude, which I have now for some time adopted, in a series of investigations undertaken in the hope of throwing some light upon nerve-function. The nature of the conditions conducive to an exhibition of increased nerve excitability, as, for instance, the increased excitability to mechanical stimuli shown during cooling of the nerve, has long convinced me of the improbability of the attempt made to explain nerve-function in terms of chemical reactions. I have therefore continuously sought for a possible physical explanation of this process. The most probable explanation, in terms of physical conditions, was an electrical one. From this point of view, the main characteristic of nerve was the number, character, and arrangement of the electrolytes contained within it. These things being unknown, remained to be discovered, but the chances of their discovery were being continually diminished by the tendency to explain all the physical phenomena of nerve in terms of functional ability and activity. To me the ability to function was equivalent to the retention of some favourable disposition of electrolytes, the act of function the motion of electrolytes.

I have therefore strongly resisted the introduction of terms conveying other than a purely physical meaning, and, most of all, have resisted the mention of the word "excitation," as affording a complete explanation of the origin of one of the most interesting physical phenomena of nerve—the injury-current. This phenomenon I carefully examined. The evidence and the arguments which had been previously made use of to associate this phenomenon with secondary consequences of injury were, as I found and explained, quite inconclusive. These arguments also were misleading, since their tendency was to detract from the interest taken in that most important feature of the nerve, its physical structure. They tended, for example, to separate this "injury-current" as a functional occurrence, from the facts of the electrical conductivity of nerve, in which I was certain lay the key to its meaning. I therefore planned the steps of my investigation on the assumption that this phenomenon was dependent upon facts entirely similar to those upon which the electrical conductivity was confessedly dependent, that is to say, upon the number, character, and arrangement of the electrolytes permanently present in the nerve. None of the relations elicited by me in the course of this investigation led me at any moment to doubt the truth of this conception. I was, however, compelled, at its close, to admit that these electrolytes were present in a more liberated condition in the neighbourhood of the injury than elsewhere. The word excitation, however, which might then have been conceivably introduced into the statement of this fact, involved so many conceptions foreign to my meaning, and possessing no relation to the facts discovered, that I carefully avoided—or rather resisted—its introduction. The fact made clear from the results of this investigation, that there were more electrolytes in motion at the site of injury than in other parts of the nerve, ran a great risk of being accepted as a proof that new electrolytes (simple waste products) were being freshly formed there as a consequence of some localised chemical change.

Now the facts are different, the actual nature of the electrolytes concerned has been in great measure determined. They have been definitely found to be of the kind suspected by me—inorganic salts, permanent constituents of the axis-cylinder. It is therefore possible to speak of this portion of the axis-cylinder in very definite terms. It is a portion containing a concentrated aqueous solution of a potassium salt, and some partially coagulated proteid, and in these conditions it is unlike other portions of the axis-cylinder. One is therefore in a position clearly to face questions asked with the object of seeking to link its existence, and the consequences of its existence, in terms of nerve-function.

Personally, I would arrange the facts in these terms. The factor in this condition of importance to the remainder of the nerve-fibre is the presence of so much potassium salt. The existence of so much potassium salt in a state of simple solution is the consequence of a violent stimulation. The consequences involved by the presence of this salt are (1) the invasion of neighbouring portions of the fibre by salt diffusing from this point, and (2) a resultant alteration in the electrical potential of points in the axis-cylinder. The direct consequence of the entrance of potassium salt, properly belonging to another portion of the fibre, is some secondary coagulation. The direct consequence of the alteration in potential is to render inexcitable the immediately neighbouring portion of the axis-cylinder, and to render more excitable more distant regions.

On the Effect of Carbon Dioxide on Geotropic Curvature of the Roots of Pisum sativum L.

By Eric Drabble, D.Sc., F.L.S. (Demonstrator in Botany, Royal College of Science), and Hilda Lake.

(Communicated by J. Bretland Farmer, F.R.S. Received May 12,—Read June 8, 1905.)

Introductory.

Many physiologists have shown that, in general, carbon dioxide exercises a narcotic or toxic influence on vegetable protoplasm, temporarily or permanently affecting its activity, according to the partial pressure under which the gas acts. De Saussure (1), as long ago as 1804, stated that, in an atmosphere containing 8 per cent. carbon dioxide, the growth of peas was less than in air; Böhm (2), in 1873, found that roots of *Phaseolus multiflorus,* after 17 days' exposure, exhibited successively less elongation in partial pressures of 2, 5, 10, and 14 per cent. carbon dioxide respectively, the temperature ranging between 17° and 19° C.; in each percentage named the growth was progressively less than in normal air. Montemartini (3), in 1892, working with roots of *Pisum,* found 7 per cent. and upwards to depress growth-activity. Chapin (4), in 1902, found the growth of roots of *Pisum sativum* and *Vicia sativa* to be diminished by 5 per cent., and arrested by 25 to 30 per cent. and upwards. Growth of the stem in the same plants was diminished by 15 per cent., and completely inhibited by 22 to 25 per cent. Experiments conducted by one of us, in conjunction with Professor Farmer, have proved that seedling peas may be kept in an atmosphere containing 20 per cent. carbon dioxide for 14 days without losing the power of renewed growth when placed in air. It is interesting to note that, in many of these plants, the plumule was destroyed, although the main root continued to grow, growth being carried on by shoots arising in the axils of the cotyledons.

Brown and Escombe (5) grew plants in increased partial pressures of carbon dioxide. The anatomy of these plants was investigated by Farmer and Chandler (6), who found the growth of the aërial parts to be diminished, while root-growth was apparently unaltered. Ewart (7) observed that carbon dioxide stops protoplasmic streaming, but he does not state the percentage employed in his experiments.

Professor J. B. Farmer and Dr. A. D. Waller, as the result of a series of experiments on the action of various substances on protoplasmic streaming in *Elodea* and *Chara*, observed that, after treatment with carbon dioxide for a short period, the subsequent rate of streaming was temporarily increased. Montemartini had, in 1892, observed that the growth in length of pea-roots was more rapid in 4 per cent. carbon dioxide than in air, and than in percentages greater than 7, but the significance of this does not appear to have been very generally appreciated. Chapin (4), in 1902, determined the optimum percentage of carbon dioxide for the growth of higher plants to be 1 to 2 per cent., and stated that, in small quantities, the effect is stimulative, whereas in large doses it acts as a poison.

Brown and Escombe's results have shown that the utilisation of carbon dioxide is, within limits, proportional to its partial pressure. In view of the facts that the weight of the plant does not increase in proportion to the absorption of the gas, it may, perhaps, be suggested that possibly a stimulating action is exercised on the protoplasm, resulting in increased photosynthesis and respiratory activity, a preponderance of the latter process explaining the absence of increase in weight.

Effect of Carbon Dioxide on Geotropism.

The fact that roots placed horizontally in boiled water do not respond to geotropic stimulus, owing to the absence of oxygen, has been known for some time. A similar failure to respond results when the roots are placed in hydrogen or other indifferent gas. That the stimulus is perceived in each case is clearly proved by the fact that, if the plant be removed from the boiled water or gas and placed vertically in air, the root-tip executes a movement out of the vertical in the direction of the previous stimulus. Czapek's ammoniacal silver nitrate and guaiacum reactions (10) seem to offer a means of demonstrating such a perception, whether the stimulus be followed by curvature or no.

In view of the fact that carbon dioxide is a protoplasmic poison, and in small doses acts as a stimulant to streaming and to growth, it occurred to us that an investigation of its action on geotropic curvatures might be not without interest. With this object, experiments were set up as follows :—

Method I.—Peas, whose radicles had emerged to a length of about 3 cm., were fixed horizontally on a strip of cork in a glass vessel, through which gases could be passed. Through one such vessel carbon dioxide was passed for various lengths of time, a second vessel being employed as an air control. While horizontal no bending took place in either set. The peas were then placed with their roots in a vertical position in air, care being taken to keep the atmosphere saturated with water vapour, and the first appearance of curvature was noted. When the stimulus was allowed to act on the horizontally placed roots for 15 minutes, no appreciable difference could be observed between the rates of curvature in the two sets when both were replaced in air at the close of the application of the stimulus. When, however, the stimulus acted for 20 minutes, in every case the curvature commenced appreciably sooner in the plants treated with carbon dioxide. The difference between the excess of bending in the plants stimulated in carbon dioxide for 15 and 20 minutes over that in their respective air controls is, perhaps, to be attributed to the slow penetration of the cells by the gas.

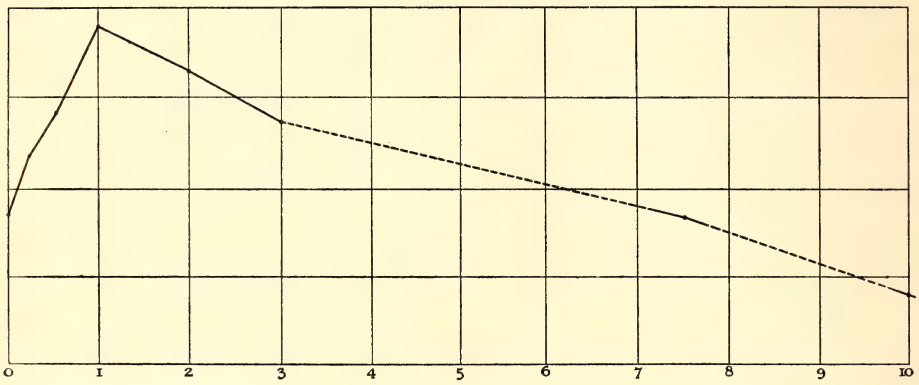
After 25 minutes' stimulation, the excess of curvature in the plants treated with carbon dioxide over that of the air-control plants was distinctly observable, though it was markedly less than in the plants treated for 20 minutes. It is probable that, during the additional 5 minutes, the carbon dioxide penetrates the cells in sufficient quantity to produce a solution in the cell-sap of supra-optimal strength, whereas in 20 minutes the optimal strength may be just attained.

Method II.—The above results seemed to be of sufficient interest to warrant a continuation of the investigation under more rigid experimental conditions. Plants, after stimulation in a horizontal position, were placed on a klinostat in such a manner that the axis of the root was parallel to the axis of rotation. The klinostat performed one revolution in 19 minutes—a sufficiently slow rate, according to Mr. Francis Darwin's (11) researches, to allow movement of the starch grains from side to side of the cell. Under these conditions, it was again found that no appreciable difference between the plants stimulated for 15 minutes in carbon dioxide and in air could be detected. After 20 minutes' stimulation, however, as before, the curvature of the roots treated with carbon dioxide was much more rapid.

Method III.—The plants were at once immersed in pure carbon dioxide, and were subsequently transferred to the klinostat. After stimulation for 20 minutes in carbon dioxide, the subsequent geotropic response set in distinctly earlier than in the air-control plants.

Method IV.—Mixtures of different percentages of carbon dioxide in air were employed with a view to determining the optimal partial pressure for geotropic response. The mixed gases were continuously passed over the peas, which were kept in a horizontal position throughout the experiment. The amount of curvature was recorded after stated intervals. By this means comparison is instituted between the curvatures due to continuous geotropic stimulus in atmospheres exerting different partial pressures of carbon dioxide. In the preceding methods the curvature was that following stimulation for limited and short periods, during which alone the carbon dioxide was allowed to act.

In order to record the curvatures observed, the following notation is adopted. The least appreciable curvature is denoted by *b*—; *b*, *b*+, and *b*++, being used to express successively greater degrees of curvature. By reading against squared paper it has been found possible very accurately to allot values to these letters. If *b*— be equivalent to a value 3, then *b* is 4, *b*+ is 5, and *b*++ is 7. By employing the values thus obtained, a curve graphically expressing our results with different partial pressures has been plotted.



Curve showing Amount of Curvature in different Partial Pressures of Carbon Dioxide. Ordinates express curvature and abscissæ percentages of carbon dioxide.

Series A.—Mixtures employed: Air; 1 per cent. CO_2 in air; 10 per cent. CO_2 in air. The response in air was more rapid than in 10 per cent. CO_2, but distinctly less rapid than in 1 per cent. CO_2 (see Table I).

Series B.—Mixtures employed: Air; 3 per cent. CO_2 in air; 7½ per cent. CO_2 in air. Curvature was effected slightly more quickly in 3 per cent. CO_2 than in air, while but little difference could be observed between the plants in air and in 7½ per cent. CO_2 (see Table II).

Series C.—Mixtures employed: Air; 1 per cent. CO_2 in air; 3 per cent. CO_2 in air. The curvature was first apparent in 1 per cent., while in 3 per cent. it appeared earlier than in air (see Table III).

Series D.—Mixtures employed: Air free from CO_2; Air; 2 per cent. CO_2. Curvature first set in in 2 per cent. CO_2, that in air being more marked than in air free from CO_2 (see Table IV).

Series E.—Mixtures employed: 1 per cent. CO_2; 2 per cent. CO_2. The first bending occurred in 1 per cent. CO_2 (see Table V).

Series F.—Mixtures employed: 0·2 per cent. CO_2; 0·5 per cent. CO_2; 1 per cent. CO_2. The earlier bendings were most vigorous in 1 per cent., those in 0·5 per cent. and in 0·2 per cent. following in decreasing order (see Table VI).

A few words of explanation may be added to the preceding account of our results. Attention was principally focussed upon the first appearance and relative magnitude of the earlier curvatures. In addition it should be noted that in CO_2 free air, in air itself, in 0·2, 0·5, 1, 2, and 3 per cent. CO_2, all the roots finally exhibited very large curvatures, while in 7½ per cent., and in 10 per cent., the actual power of response seemed to be not only delayed, but actually diminished to a very large degree.

Throughout the experiments the range of temperature did not exceed about one degree Centigrade, namely, from 15° to 16°, and in every case any such small range was exactly similar for each set of peas used in comparison.

A few representative tables have been selected, and are given below:—

Table I.

Per cent. CO_2.	No. of peas.	Curvature after 45 mins.	1 hr. 15 mins.	1 hr. 45 mins.	2 hrs. 15 mins.	2 hrs. 45 mins.
Air	6	0	3*b* –	*b* +, *b*, 2*b* –	*b* +, *b*, 3*b* –	2*b* + +, 2*b* +, *b*
1.........	6	3*b* –	2*b*, 3*b* –	2*b* +, *b*, 3*b* –	2*b* +, 2*b*, 3*b* –	*b* + +, 2*b* +, 2*b*
10.........	6	0	0	2*b* –	3*b* –	3*b*, 2*b* –

Table II.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 30 mins.	2 hrs.	3 hrs.
Air	8	0	2*b* –	3*b* –	2*b* +, *b*, 4*b* –	*b* + +, 4*b* +, 2*b*, *b* –
3	8	0	3*b* –	3*b* –, *b*	*b* +, 2*b*, 3*b* –	3*b* + +, *b* +, 2*b*, *b* –
7½.........	8	0	2*b* –	2*b* –	*b*, *b* –	3*b*, 2*b* –

Table III.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 30 mins.	2 hrs.	3 hrs.
Air	6	*b* –	2*b*	3*b*, *b* –	2*b* +, *b*, 2*b* –	4*b* +, *b*, *b* –
1	5	2*b* –	2*b*, 2*b* –	2*b* +, 2*b*, *b* –	3*b* + +, *b* +, *b*	4*b* + +, *b* +
3	6	*b* –	*b*, 3*b* –	*b* +, 3*b*, 2*b* –	*b* + +, 4*b* +, *b* –	2*b* + +, 4*b* +

Table IV.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 45 mins.	2 hrs. 15 mins.
0...........	8	3*b* –	5*b* –	3*b*, 3*b* –	*b* +, 2*b*, 3*b* –
Air........	8	0	*b*, 3*b* –	*b* +, 2*b*, 5*b* –	3*b* +, 2*b*, 3*b* –
2...........	8	*b*, *b* –	2*b*, 4*b* –	2*b* +, 3*b*, 3*b* –	*b* + +, 2*b* +, 4*b*

Table V.

Per cent. CO_2.	No. of peas.	Curvature after 1 hr. 15 mins.	1 hr. 45 mins.	2 hrs. 30 mins.	3 hrs.
1...........	12	2*b*, 6*b* –	8*b* +, 2*b*, 2*b* –	*b* + +, 8*b* +, 3*b*	3*b* + +, 8*b* +, *b*
2...........	12	8*b* –	6*b* +, 2*b*, 4*b* –	9*b* +, 2*b*, *b* –	*b* + +, 10*b* +, *b* –

Table VI.

Per cent. CO_2.	No. of peas.	Curvature after 30 mins.	1 hr.	1 hr. 30 mins.	2 hrs.	2 hrs. 30 mins.
0·2	12	0	4*b* –	3*b*, 6*b* –	*b* +, 5*b*, 5*b* –	5*b* +, 6*b*
0·5	12	0	6*b* –	5*b*, 4*b* –	3*b* +, 5*b*, 3*b* –	*b* + +, 6*b* +, 3*b*, *b* –
1·0	12	0	*b*, 7*b* –	2*b* +, 6*b*, 3*b* –	8*b* +, 3*b* –	*b* + +, 9*b* +, *b*, *b* –

In considering the mode of action of carbon dioxide several possibilities present themselves. It is conceivable that increase of the partial pressure of carbon dioxide above a certain point, by the mere presence of an increased number of molecules of this substance, may interfere with the liberation of more carbon dioxide in the ordinary respiratory function, so depressing the activity of the cell. On the other hand, or in addition to this mode, the carbon dioxide may exert a direct action on the protoplasm itself. Although this seems to have been the generally accepted explanation, it appears to afford but a very imperfect picture of the true course of events. Recent work on the action of poisons suggests with considerable force that in many cases dissociation of the molecule precedes the manifestation of physiological effect. Walker (12) has shown that when carbon dioxide is dissolved in water, a solution containing unchanged CO_2, H_2CO_3 and dissociated H_2CO_3 (H^+ and HCO_3^-) is produced, the proportion of the dissociated substance depending on the strength of solution.

It is not improbable that the dissociated substance is principally concerned in the causation of the physiological effects described above. The manifestation of these results may be due to the action of the H, or of the HCO_3 ions. (The possibility of direct action of H_2CO_3 being of the same order as that of the unchanged CO_2.) It is hoped shortly to publish the results of experiments undertaken with a view to the elucidation of this at present obscure problem.

Whether the action take place by union with the protoplasm itself, or by combination with some metabolite, thereby putting this latter substance out of the field of physiological action, remains to be determined.

It must be borne in mind that the external partial pressure of carbon dioxide alone is known, whereas it is the partial pressure within the pea that determines the strength of solution and the amount of ionisation. It is true that the external partial pressure to a very considerable extent determines the internal pressure, but in addition to this is the carbon dioxide constantly evolved in respiration of the cells. At a certain stage the carbon dioxide penetrating from without, together with that evolved by the cells, will create an internal partial pressure equal to the external one. But as respiration proceeds the partial pressure within the pea will exceed that outside, and a slightly higher internal pressure will be maintained as long as respiration continues. Hence, what has been determined by the above experiments is the partial pressure of carbon dioxide in the external atmosphere sufficient to produce during the time of experiment the optimum strength of solution in the cell sap.

LITERATURE.

1. De Saussure. "Recherches chimiques sur la végétation," Paris, 1804, in 'Ostwald's Klassikern.'
2. Böhm, Jos. "Sitzungsberichte der Wiener Akademie," 1873.
3. Montemartini. "Sulla influenza di atmosfere ricche di biossido di carbonio sopra lo sviluppo e la struttura delle foglie," 'Atti del Istituto Botanico di Pavia,' 1892.
4. Chapin, P. "Einfluss der Kohlensäure auf das Wachsthum. Flora," 1902.
5. Brown and Escombe. "The influence of varying amounts of Carbon dioxide in the air on the photosynthetic process of leaves and on the mode of growth of Plants," 'Roy. Soc. Proc.,' London, 1902.
6. Farmer, J. B., and S. E. Chandler. "On the influence of an excess of Carbon dioxide in the air on the form and internal structure of plants," 'Roy. Soc. Proc.,' London, 1902.
7. Ewart, A. "The Physics and Physiology of Protoplasmic Streaming," London, 1903.
8. Farmer, J. B., and A. D. Waller. "Observations on the action of Anæsthetics on vegetable and animal Protoplasm," 'Roy. Soc. Proc.,' London, 1898.
9. Brown and Escombe. "Static Diffusion of Gases and Liquids in relation to the assimilation of Carbon in Plants." 'Phil. Trans.,' B, 1900.

10. Czapek, F. "The Antiferment Reaction in Tropistic Movements of Plants," 'Annals of Botany,' 1905.
11. Darwin, O., and Pertz, D. F. M. "Note on the Statolith Theory of Geotropism. 1. Experiments on the Effects of Centrifugal Force." 'Roy. Soc. Proc.,' June, 1904.
12. Walker, J. "The state of Carbon Dioxide in Aqueous Solution," 'Journ Chem. Soc., London, 1903.

Preliminary Note on the Occurrence of Microsporangia in Organic Connection with the Foliage of Lyginodendron.

By R. Kidston, F.R.S.L. & E., F.G.S.

(Received May 25,—Read June 8, 1905.)

(Plate 6.)

Any certain knowledge at present possessed of the fructification of the *Pteridospermeæ** is restricted to the female organ or seed. Suggestions have been made that the microsporangia of *Lyginodendron Oldhamium*, the *Sphenopteris Höninghausi* of Brongniart, might be found in *Telangium Scotti*,† but the evidence for this was chiefly its association with fragments of *Lyginodendron Oldhamium*. From the structure of *Telangium Scotti*, I am satisfied that it cannot be the microsporangia of *Sphenopteris Höninghausi*, and in any case the organic connection was not demonstrated.

Among some specimens from the 10-foot ironstone measures (Westphalian, series), Coseley, near Dudley, sent me for examination by Mr. H. W. Hughes, F.G.S., were a number of examples of *Sphenopteris Höninghausi* preserved in small nodules.

Many of these were fragments of barren pinnæ, but a few showed a fructification referable to *Crossotheca*, Zeiller,‡ in organic connection with barren foliage of *Sphenopteris Höninghausi*, while other specimens consisted of fertile pinnæ or portions of pinnæ unassociated with any barren pinnules. Their identity with the fertile pinnules found in connection with sterile ones, leaves no doubt as to their also belonging to *Sphenopteris Höninghausi*.

I do not propose to enter into a detailed account of the structure of the

---

* Oliver and Scott, "On the Structure of the Palæozoic Seed *Lagenostoma Lomaxi*, etc.," 'Phil. Trans.,' ser. B, vol. 197, p. 239, 1904.

† M. Benson, *Telangium Scotti*, a new species of *Telangium* (*Calymmatotheca*), showing structure, 'Ann. of Botany,' vol. 18, p. 161, Pl. 11, 1904.

‡ *Crossotheca*, Zeiller, 'Ann. d. Sc. Nat., 6^e sér., Bot.,' vol. 16, p. 180, Pl. 9, figs. 1—9, Aug., 1883, "Flore foss. Bassin houiller d. Valenciennes," p. 33, fig. 21, 1888.

Microsporangia of *Sphenopteris* (*Crossotheca*) *Höninghausi* (= *Lyginodendron Oldhamium*) in the present note, but merely wish to record the organic connection observed, and to give a brief description of their chief features. Their full description will be reserved for another communication.

The fertile pinnule is oval, entire, and attached to the rachis by a stout pedicel, which thickens very slightly upwards before merging into the pinnule, to the upper surface of which it appears to be united for a short distance. The pinnules seem to have been thick in substance, and the vascular trace enters it from the pedicel a short distance from the margin, where it immediately divides into two main branches which separate slightly from each other. Lateral veinlets probably existed, but they have not been observed in the fertile pinnules of *Sphenopteris* (*Crossotheca*) *Höninghausi*, though indications of their presence are clearly seen in the fertile pinnules of a closely allied but undescribed species.

Each fertile lobe bore six to eight broadly lanceolate sharply-pointed microsporangia. In the early condition the sporangia are bent inwards, and form a small hemispherical bunch with their apices meeting in the centre. At maturity the sporangia spread outwards, when they appear as a fringe hanging from the margin of the fertile pinnule, but are in reality connected for some distance to its lower surface. The microsporangia are bilocular, the parallel loculi being only separated by a narrow band of tissue. Dehiscence took place by a longitudinal cleft which passes down the inner surface of the sporangium in the line of the dividing wall of the two loculi.

Many of the sporangia still retain the microspores, which are easily removed for microscopical examination. They are circular or slightly oval, and measure 50 μ to 57 μ in diameter. Their outer surface is granular, from the presence of very minute blunt points, and is also provided with a triradial ridge, which, however, is seldom clearly seen on account of the cell wall being crumpled into ridges—probably the result of contraction.

A few examples of *Crossotheca Höninghausi* are figured on the accompanying Plate, after photographs by the author. Fig. 1 shows a fragment of a barren pinna, enlarged two times, to illustrate the pinnule cutting of the species. A specimen showing the *Crossotheca* fructification in organic connection with the barren pinnules, enlarged two times, is given at figs. 2 and 3. These two figures show the impression of the plant on the two surfaces of the split nodule. All the lateral pinnæ show some fertile pinnules on their upper portions, while sterile ultimate pinnæ are seen at the base of the penultimate pinnæ. These are especially well seen at *c* and *d*, but sterile pinnules also occur on *b*, and at the apex of the specimen.

A small fertile pinna is given natural size at fig. 4, and the same specimen is enlarged two times at fig. 5, where the arrangement of the microsporangia is clearly exhibited.

My thanks are due to Mr. H. W. Hughes, F.G.S., to whom I have been so often indebted in the past for assistance in my studies of the Carboniferous Flora, for the opportunity of describing these interesting specimens.

On the Efferent Relationship of the Optic Thalamus and Deiter's Nucleus to the Spinal Cord, with special reference to the Cerebellar Influx Theory (Hughlings Jackson) and the Genesis of Decerebrate Rigidity (Sherrington).

By F. H. THIELE, M.D., B.Sc., M.R.C.P., Pathologist to University College Hospital.

(Communicated by Sir Victor Horsley, F.R.S. From the Laboratory of Chemical Pathology, University College, London. Received April 19,—Read May 18, 1905.)

In the following experiments it was determined to re-investigate the matter of the thalamo-spinal mechanism from the point of view of tracing the relations of the thalamus to the mesencephalon and hind-brain.

Now, the thalamic grey centres are in association with the bulb and cord by the thalamo-bulbar and spinal tracts, placed on and around which are the rubro-spinal, tecto-spinal, Deiter-spinal, and the lateral cerebello pontine tracts. As the pyramidal fibres run through the mesencephalon it became necessary to exclude them in arranging the investigation.

The general methods employed were as follows:—

A. The localisation of the genesis of decerebrate rigidity and the influence of the cerebellum were determined by making successive coronal sections through the thalamus, mesencephalon pons, and bulb.

B. Excitation of the superior and middle cerebellar peduncles in normal animals and in others in which the pyramidal tracts had been previously degenerated by suitable lesions in the middle zone of the cerebral hemispheres.

C. Excitation of the cut surface of the thalamus and mesencephalon with or without previous pyramidal degeneration.

The experiments were all performed under complete anæsthesia, the anæsthetics used being chloroform or ether. In cases where the brain was

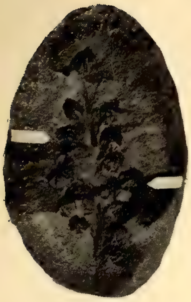


FIG. 4. $\frac{1}{1}$.

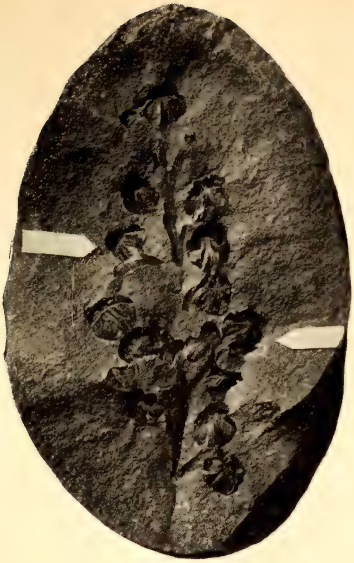


FIG. 5. × 2.

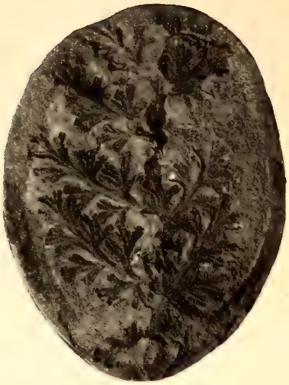


FIG. 1. × 2.

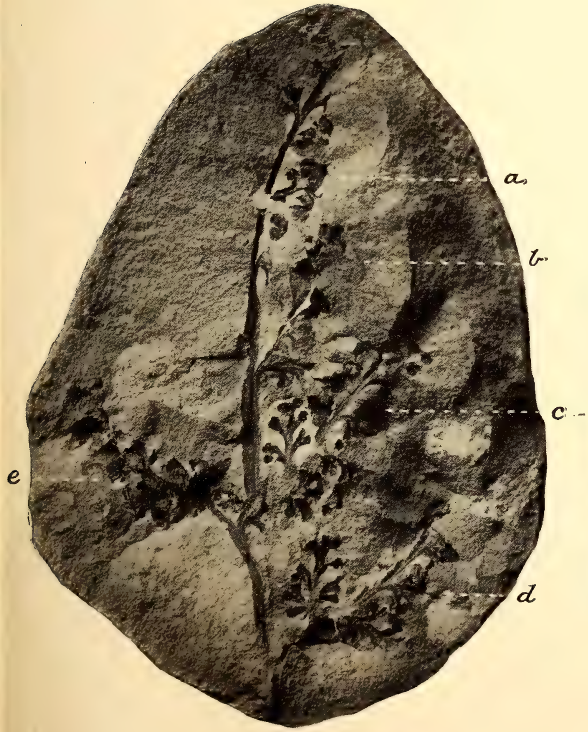


FIG. 2. × 2.

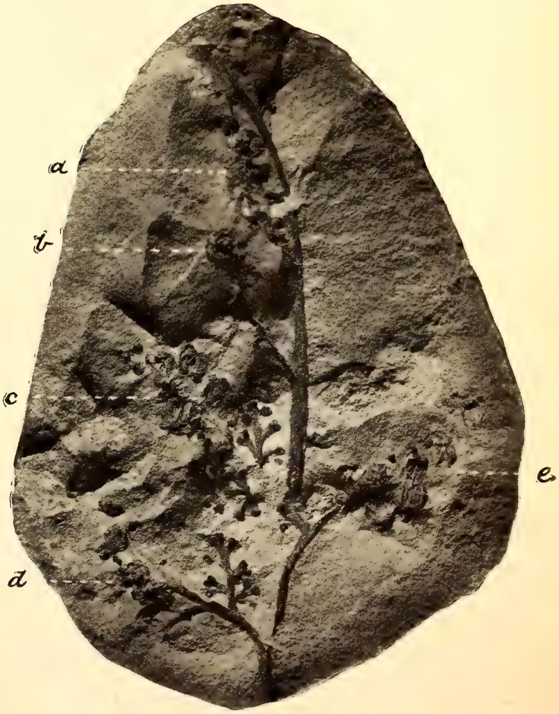


FIG. 3. × 2.

Crossotheca (*Sphenopteris*) *Höninghausi*, Brongt.

removed in slices the carotids were previously tied on both sides. The animals used were cats, dogs, and monkeys.

The following results were obtained:—

A. *Those Relating to Decerebrate Rigidity.*

It was found that rigidity did not commence till the section was made at the level of the posterior part of the optic thalamus. The rigidity thus obtained became more intense as the lesion was carried farther back in the mesencephalon. The rigidity lasted till the line of section passed through the trapezium. Directly the section passed behind the pons the rigidity disappeared, and did not return as long as the animal was kept alive. In some cases at this stage the animal assumed a general position of flexion for a short time. Gradual removal of the cerebellum was found to be without influence in decerebrate rigidity when this had been previously produced, but immediately the lesion was carried through Deiter's nucleus the rigidity was abolished. This abolition of the rigidity was homolateral.

Similarly division of the Deiter spinal tract without any injury to the rest of the cerebellum caused homolateral relaxation of decerebrate rigidity. In some cases, however, the relaxation was not complete, or it returned to a slight degree after a time. In such exceptional cases subsequent unilateral bulbar transection did not always abolish the persistent rigidity, which then must clearly be of spinal origin. An irritative lesion of Deiter's nucleus or the tract caused a temporary increase of the spasm.

When unilateral lesions were made in the mesencephalon the results varied with the position of the section. If the lesion was made immediately behind the thalamus, contralateral rigidity was by far the most marked; if farther back, the homolateral rigidity was most marked.

Section of the afferent roots belonging to a limb caused the abolition of the decerebrate rigidity in that limb and prevented its occurrence when performed previous to the mesencephalic transection. Division of the posterior columns or of the direct cerebellar tracts, or of both, in no way interfered with the occurrence of decerebrate rigidity or caused its abolition.

In all the experiments in which removal of the tentorium was required marked extensor rigidity exactly like that following mesencephalic transection occurred. The same result was invariably obtained, but the more carefully the removal was accomplished the less marked was the rigidity.

Bisection of the superior vermis without previous removal of the tentorium also produced a bilateral extensor rigidity exactly like decerebrate rigidity.

Removal of one lateral half of the posterior part of the superior vermis also caused a bilateral extensor rigidity most marked homolaterally.

Removal of the cerebellar hemispheres by successive sagittal slices without previous removal of the tentorium always caused a bilateral extensor rigidity. With the removal of only a small portion the rigidity was slight, but with the subsequent removals it became much greater.

When, however, nearly the whole of one cerebellar hemisphere had been removed the homolateral rigidity became very much less, but remained in full intensity on the contralateral side. When the lesion included Deiter's nucleus the homolateral rigidity completely disappeared, and when the mesencephalon was subsequently transected there was no recurrence of rigidity on the homolateral side.

Removal of the vermis and the cerebellar hemispheres by horizontal slices caused bilateral extensor rigidity, which remained till Deiter's nuclei were involved in the lesion. Removal of the cerebellum in this way had no influence on existing decerebrate rigidity, which, however, became abolished when Deiter's nucleus was destroyed.

Stimulation of the vermis and the dorsal surface of the cerebellar hemispheres caused relaxation of the spasm in decerebrate rigidity. The relaxation was most marked on the homolateral side.

The results obtained in this way were exactly the same whether the pyramidal tracts had previously been removed by degeneration or not.

Sections of the superior cerebellar peduncle had only a very slight effect as regards rigidity. In this experiment it was always necessary to remove part of the tentorium, and no matter how carefully this was performed, a certain amount of rigidity supervened. Section of the superior peduncle caused the rigidity to become slightly increased. Subsequent mesencephalic transection in front of the line of section of the peduncle caused the rigidity to become greatly increased.

The effects of section of the superior cerebellar peduncles and of piecemeal removal of the cerebellar hemispheres on the knee-jerks were also noticed.

Section of the superior peduncle on one side, directly after emerging from the cerebellum, caused the homolateral knee-jerks to become increased. Piecemeal removal of the cerebellum by sagittal slices, when about one-third of the lateral lobe had been removed, caused in cats an increase in the homolateral knee-jerk, the contralateral jerk remaining the same or being slightly increased. More extensive removal increased the knee-jerks on both sides, the homolateral being brisker than the contralateral. Complete removal, including Deiter's nucleus, produced no further change.

In dogs, piecemeal removal of one cerebellar hemisphere produced the same results as obtained by Dr. Risien Russell, namely, increased jerks on the homolateral side, diminished on the contralateral. This did not occur till about one-half of one lobe had been removed, and further removal had no other effect. Removal of one lateral half of the posterior part of the superior vermis caused an increase in the homolateral knee-jerks.

In both cats and dogs injury to other parts of the mesencephalon, and simply scratching the superior cerebellar peduncle, caused the homolateral knee-jerks to become increased.

The results of these experiments can be summed up as follows:—

1. Decerebrate rigidity does not commence till the lesion passes through the posterior part of the thalamus and is independent of any injury to the pyramidal system.

2. The inhibitory centre lies in the thalamus, the control is a crossed one, and the decussation takes place high up.

3. The cerebellum has no influence on decerebrate rigidity; the adjuvant centre is Deiter's nucleus.

4. Decerebrate rigidity does not appear to be due to the interruption of the afferent channel of the cerebro-cerebellar circuit.

5. The tonic condition depends upon the reflex arc being intact; injury to the posterior columns or the direct cerebellar tract has no effect.

6. A condition of extensor rigidity occurs after partial or complete removal of the tentorium, bisection of the vermis, injury to the vermis, or partial cerebellar ablation.

The results as regards the knee-jerks agree with those obtained by Risien Russell in dogs, and by Ferrier and Turner in cats and monkeys. Complete removal of one hemisphere, however, is not necessary to produce the changes.

B. *The Results obtained by Stimulation of the Middle and Superior Cerebellar Peduncles.*

In order to expose the peduncles of the cerebellum the posterior part of the calvarium, the tentorium, and the greater part of the posterior fossa on one side were removed, the occipital lobe was drawn up, and the cerebellum drawn back to expose the middle peduncle. Stimulation of the middle peduncle produced bilateral, facial, and nasal movements most marked homolaterally. The trapezii and pectorals of both sides were affected, the fore-limbs were protracted at the shoulder and flexed at the elbow, the hind-limbs were slightly flexed and drawn up. The back muscles were also thrown into a state of contraction, the effect in all parts being especially homolateral. Eye movements also occurred. They were, however, very various, usually movement towards the homolateral side.

Stimulation of the superior peduncle was not very often successful; in the majority of cases the results were the same as for the stimulation of the middle peduncle. These results occurred whether the pyramidal tracts were intact or degenerated.

From these experiments it appears that the cerebellum by way of its peduncles exercises a control over the skeletal muscles, the control being chiefly over the muscles of the trunk and girdles.

During the course of these experiments stimulation in the neighbouring regions of the lateral fillet, posterior corpus quadrigeminum, and IVth nerve produced the following results:—

Stimulation of the IVth nerve produced movement of the homolateral eyes upwards and outwards.

Stimulation of the lateral fillet produced pricking of and rotation of the opposite ear outwards and backwards, conjugate deviation of the eyes to the contralateral side.

Stimulation of the lateral aspect of the posterior corpus quadrigeminum produced no result. Vocalisation was not noted, since the animal was anæsthetised through a tracheotomy tube.

C. *Results obtained by Stimulation of the Cut Surface of the Optic Thalamus and Mesencephalon.*

It was only when the posterior part of the optic thalamus was stimulated that any results were obtained.

Stimulation in this region of what appeared to be the median nucleus produced very definite motor phenomena. The animal presented a certain degree of decerebrate rigidity, stimulation produced retraction and flexion of the homolateral fore-limb, protraction and extension of the contralateral fore-limb. The homolateral hind-limb was in some cases extended, the contralateral flexed. In other cases the result was to produce a flexion of both hind-limbs.

There was also usually contraction of the trunk muscles, most marked on the homolateral side, causing in some cases rotation, so that the homolateral shoulder became the lower.

This result was obtained in cats and monkeys, and was quite independent of the pyramidal tracts, since it was always obtained in those cases where the pyramidal tracts had been eliminated by degeneration previously. Similar results were obtained when the cut surface of the mesencephalon was stimulated at different levels. The area from which these movements were obtained was smaller than that in the thalamus. The tract appeared to be the rubro-spinal tract or one coming down with it. The tract was traced as far as the medulla.

The strength of faradic current required to produce these movements was weaker than that necessary to elicit pyramidal movements. Continued stimulation produced coarse clonus.

Stimulation of the cut end of the posterior longitudinal bundle at the various levels of the sections produced no result in any case.

It will be seen from these results that the movements elicited by stimulation of the posterior end of the optic thalamus are co-ordinated movements affecting the whole body, and are the same as those of walking.

It would thus appear that there exists in the posterior part of the optic thalamus a centre which controls the ordinary act of walking, and the path along which impulses are carried is the rubro-spinal tract.

These results are in accordance with those of Goltz, who removed the cerebral hemispheres in dogs, and those of Brown-Sequard, Starlinger, Probst, and Rothmann, who showed that there was another motor tract beside the pyramidal as the result of cortical stimulation after the pyramidal fibres had been made to degenerate.

It seems justifiable to draw the following conclusions as the results of these experiments:—

1. The anterior cornual cells are under the control of the optic thalamus and Deiter's nucleus, the former exerting an inhibitory action, the latter an adjuvant action. The influence of Deiter's nucleus is also controlled to some extent by the cerebellar cortical cells. The thalamic control is crossed; the cerebellar influence is homolateral.

2. Both the thalamus and cerebellum exert a motor control over the muscles of the body. The cerebellar influence is chiefly on the trunk and girdle muscles, and is preponderatingly homolateral.

3. In the thalamus is a centre which controls the co-ordinated movements of locomotion. The path along which the control is exerted is probably the rubro-spinal tract.

4. The afferent and efferent cerebro-cerebellar channels appear to function as channels of communication between the cerebrum and cerebellum to produce co-ordination.

In the maintenance of the muscular tonus, the reflex arc is necessary. Section of the afferent channels in the cord has no result on the muscular tonus.

In conclusion, I must state that this work owes its inception to Sir Victor Horsley, to whom I owe very much for his great kindness and constant advice during the progress of this investigation.

On Endophytic Adaptation shown by Erysiphe Graminis *DC. under Cultural Conditions.*

By Ernest S. Salmon, F.L.S.

(Communicated by Professor H. Marshall Ward, F.R.S. Received February 24,—Read April 6, 1905.)

(Abstract.)

In recent papers by the author the fact has been pointed out that certain species of the *Erysiphaceæ* are able, under cultural conditions, to infect their host-plants vigorously when their conidia or ascospores are sown on the cells of the internal tissues exposed by means of a wound, although the fungi in question are confined normally to the external surface of the epidermal cells.

The present paper gives the results of investigations carried out in the laboratory of Professor Marshall Ward at Cambridge, with the object of ascertaining the details of growth of the fungus under these abnormal conditions, and of discovering to what extent the hyphæ penetrated into the intercellular spaces of the internal tissues, and whether haustoria (normal or otherwise) were produced by these hyphæ.

A rapid survey is first made of our present knowledge of the mycelial characteristics of the *Erysiphaceæ* in relation to their parasitic habit. The species of the *Erysiphaceæ* were regarded since De Bary's time as strict ectoparasites, until in 1899 Palla discovered the semi-endophytic habit of the genus *Phyllactinia.* With this exception the species of the *Erysiphaceæ,* so far as they have been investigated, have been found to be strictly ectoparasitic in habit, the hyphæ of the mycelium being confined to the external surface of the epidermal cells (never gaining access to the intercellular spaces of the internal tissues), and merely sending haustoria either into the epidermal cells alone, or, in the case of one species, into the subepidermal cells as well.

The experiments carried out, and the methods employed in the present investigations are then described. The fungus used was the conidial stage of *Erysiphe Graminis* DC., a strict ectoparasite under normal circumstances. Young leaves of oats and barley were cut off from seedling plants, and a minute piece of tissue was cut out with a sharp razor from the upper surface of the leaf. In this operation the upper epidermis was removed, and often a considerable amount of the mesophyll also, so that in inoculation

the conidia were sown on the sub-epidermal or deeper layers of the exposed mesophyll, or even on the internal surface of the lower epidermis. After inoculation, the leaves were placed on damp blotting-paper in a Petri dish. By the sixth to eighth day vigorous infection had nearly always resulted, the surface of the wound bearing patches of clustered conidiophores. The leaves were then fixed in Flemming's fluid or in chromacetic, and subsequently embedded in paraffin, microtomed, and stained with Diamant fuchsin and Lichtgrün.

It was found on examining such wounded leaves that the fungus had invaded the internal tissues to a remarkable extent. Where the mesophyll-cells remaining uninjured were several layers deep, the hyphæ had penetrated inwards, winding through the intercellular spaces as far as the internal surface of the lower epidermis. Haustoria were sent into the cells of the superficial layer of the mesophyll by the hyphæ creeping on the surface of the wound, and into all the deeper layers of the mesophyll by the hyphæ running in the intercellular spaces. The cells of the lower epidermis were also attacked, the internal wall having been penetrated. The sheath-cells of the vascular bundles were much invaded by very vigorous haustoria. The haustoria formed in the cells of the internal tissues resemble in every way those which occur normally in the epidermal cells.

The hyphæ enclosed in intercellular spaces, either just below the surface of the wound or deep down in the internal tissues, struggle to produce conidiophores. The respiratory cavities over the stomata of the lower epidermis were in a great number of cases full of vigorous hyphæ producing young conidiophores. When the intercellular space, where the young conidiophore was produced, was shut off from the open air by only a thin membrane consisting of the walls of collapsed mesophyll-cells, the young conidiophore growing upwards, sometimes proved able to break through it and continue its growth. The direction of growth of the young conidiophores produced in the respiratory cavities and other intercellular spaces was usually vertical, and towards the surface of the wound. Examples were observed, however, of young conidiophores growing horizontally in intercellular spaces between the mesophyll-cells, or, in a few cases, vertically, with the apex of the conidiophore directed away from the surface of the wound.

In several cases hyphæ had penetrated laterally, in a direction parallel to the surface of the leaf, from the edge of the wound, and occurred in the intercellular spaces in the middle of the mesophyll, at places where all the tissues, including the epidermis above and below, were uninjured. In such places both haustoria and young conidiophores were produced.

Figures are given illustrating the details of the growth of the hyphæ in the interior of the leaf, and the production of haustoria and intercellular conidiophores.

The author, reviewing the results of the investigations, points out that they afford proof that *E. Graminis* is not, as perhaps might have been expected, so highly specialised as an ectoparasite as to be necessarily restricted for its food-supply to cells of the epidermis; but shows itself capable of immediate adaptation to conditions closely resembling those obtaining in endophytism.

This fact suggests the possibility that under some circumstances the mycelial hyphæ of species of the *Erysiphaceæ* which are normally ectoparasites may penetrate into the internal tissues of their host-plants exposed through wounds caused in nature by the attacks of animals or by physical agency It is pointed out, however, that the successful entry of the hyphæ might be prevented, either by the drying up of the superficial layers of cells, or by the healing processes shown by many actively growing leaves.

A Preliminary Communication on the Life History of Trypanosoma balbianii.

By W. S. Perrin, B.A., Shuttleworth Research Student of Gonville and Caius College, Cambridge.

(Communicated by A. Sedgwick, F.R.S. Received May 1,—Read June 8, 1905.)

Trypanosoma balbianii, Certes, is a primitive member of the family of the Trypanosomidæ, and occurs in great numbers as a parasite in the gut of the oyster, where it may be present, either swimming freely in the fluid contents or enclosed within the crystalline style, when this structure is present. The species has been worked at by Certes* and Lustrae,† the latter of whom describes the presence of an undulating membrane and the external features of the process of division. The nucleus is, however, described by neither author.

The material used in prosecuting this research was obtained from oysters fished from the Adriatic Sea, off the coast of Rovigno, but the parasite also occurs in the oysters of Schleswig-Holstein and the north coast of France. The distribution is thus a wide one. The research has been carried out under the guidance of Dr. von Prowazek, my great indebtedness to whom I here take the opportunity of expressing.

---

* Certes, 'Bulletin Soc. Zool. France,' vol. 7, 1882, p. 347; *ebenda*, vol. 16, 1891, p. 95.

† Lustrae, 'Actes d. l. Soc. Linn. Bordeaux,' vol. 50, 1896, p. 265.

Structure.—Exclusive of the male and female gametes, *Trypanosoma balbianii* occurs in two modifications, which will be referred to as the indifferent and female forms.

The Indifferent Form (fig. 1).—This is so named as it exhibits no sexual characters, and appears to give origin, directly or indirectly, to all the other three forms—to the male gamete and female form directly, and to the female gamete indirectly through the agency of the female form.

In outward appearance the indifferent form is an elongated snake-like structure varying from about 26 μ to 100 μ in length, and from about 0·5 μ to 3 μ in breadth. The diameter is practically uniform throughout, the two extremities being rounded off. The body of the cell consists of a homogeneous non-granular mass of protoplasm enclosed within a tough pellicule, the periplast, which consists of a number of longitudinally-arranged fibrillæ embedded in a structureless matrix. These fibrillæ are probably the active agents in producing the vigorous and multiform contractions by which the animal propels itself through the fluid medium in which it lives. A nucleus is present, typically in the form of a spirally-wound band, which extends practically from one end of the animal to the other, only a small portion of the creature, from 2 μ to 3 μ in length at each extremity, being free from it. In the resting state the nuclear band is apparently homogeneous, but during longitudinal division it can be seen to become differentiated into a spirally-wound feebly-staining thread with masses of chromatin arranged along the sides at regular intervals (fig. 2, 2). The central thread is probably homologous with the karyosom of other Trypanosomes, while the chromatin masses correspond to the rest of the main nucleus. As will be seen later, this central thread becomes again differentiated from the rest of the nucleus during the encystment of the female forms. The nuclear band is thus probably to be

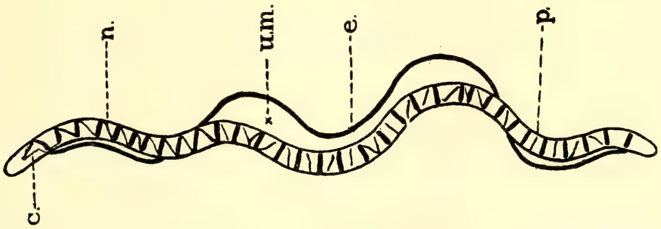


Fig. 1.—Diagrammatic representation of indifferent form. n., nucleus in the form of a homogeneous spirally-wound band. u. m., undulating membrane. e., thickened edge of und. mem. p., periplast. c., chromatin thread connecting nuclear band and thickened edge of membrane.

regarded as consisting of two parts, a central rod-like karyosom and a peripheral mass of chromatin, which in the normal resting nucleus masks the karyosom.

An undulating membrane may or may not be present, the variability of this structure being particularly striking. When present it is wound spirally round the body of the Trypanosome, extending to the same distance as the nucleus at the ends. In favourable specimens the undulating membrane can be seen at one end of the animal to be connected with the nucleus by a fine thread of substance, staining like chromatin (fig. 1, *c*). At the other end the nucleus is apparently not continuous with the undulating membrane. The edge of the membrane is thickened, and stains like chromatin. This feature, together with the fact that the edge of the membrane is connected at one end with the nuclear band, render it probable that the membrane, at any rate in part, is a development of the nucleus. Flagella and blepharoplast are absent. Motion takes place in either direction with equal facility, and the anterior and posterior ends cannot be distinguished.

The indifferent forms undergo multiplication by longitudinal division within the substance of the style, and where this process has reached such a stage that the style is closely packed with the parasites, they commence to encyst at the periphery of the style, the cysts passing out from the gut into the sea to infect fresh hosts. Where the oyster is kept out of water, the indifferent forms either encyst or undergo continued multiplication by longitudinal division, whereby they suffer great diminution in thickness, becoming in many cases mere threads, scarcely visible under the very highest powers of the microscope.

The Female Forms.—These are so named because, from a consideration of their structure and behaviour, it seems highly probable that they are the precursors of the female gametes. They differ from the indifferent forms in their stouter build, thicker periplast and undulating membrane, and occasional presence of vacuoles. Structurally they grade into the indifferent forms, and it is only in their extreme state of development that the two modifications can be distinguished from one another. In their behaviour, when subjected to adverse conditions of existence, however, the females exhibit a striking difference to the indifferent forms. In the first place they are much more resistent, neither immediately undergoing encystment nor entering into a phase of accelerated reproductive activity on the initiation of unfavourable conditions of life; and in the second place, when encystment does at last occur, they afford evidences of a parthenogenetic process in their nuclear changes, evidences lacking in the encystment of the indifferent forms. From the above features of their structure and behaviour, these forms appear to deserve to be regarded, provisionally at any rate, as being of female character, although the intermediate stages between them and the female gamete have not been obtained. In the indications it affords of the existence of indifferent and female forms, *Trypanosoma balbianii* resembles *Trypanosoma noctuæ* described by Schaudinn.*

The question of the relationships of *Trypanosoma balbianii* with other Trypanosomes will be discussed in the complete paper. It is here only necessary to point out that *Trypanosoma balbianii* is by far the most primitive of all Trypanosomes known. In many points it realises the "*Urhæmoflagellat*" imagined by Schaudinn as the ancestor of the Trypanosomidæ, though upon the whole it is of yet more simple structure. It is interesting also to note that in the distribution of its nuclear material through the cell in the form of a long spiral thread, *Trypanosoma balbianii* presents a condition not far removed from those bacteria which possess a nucleus in form of small masses of chromatin scattered through the protoplasm of the cell.

Longitudinal Division is the normal method of multiplication for both female and indifferent forms. Division is begun by the undulating membrane dividing into two longitudinally, the body following suit. The body does not, however, divide completely, the split which forms not extending quite up to one extremity. It thus happens that the two halves, after separating from one another through an angle of 180°, remain attached at the ends, producing the appearance of a Trypanosome twice the length of an ordinary individual, which, after a period of considerable activity—the two halves endeavouring to wriggle apart—divides into two. A transverse division is thus simulated in the last stage of the process of longitudinal division. The nuclear band meanwhile undergoes a series of remarkable changes, whereby its division into two equal parts is effected. The homogeneous band resolves itself into a spirally-wound thread, along which the remainder of the chromatic nuclear substance is arranged in small masses placed at more or less regular intervals (fig. 2, 2). The spiral thread, with its masses of chromatin, next condenses to form a straight homogeneous rod, which breaks up into segments (fig. 2, 3, 4). These segments again divide into a number of small dumb-bell shaped rods (fig. 2, 5), which, by transverse division, give rise to a number of approximately spherical nuclear masses, the chromosomes (fig. 2, 6). Each chromosome finally divides into two (fig. 2, 7), in a plane containing the long axis of the Trypanosome, a double row of chromosomes extending through the whole length of the creature being thus produced. The longitudinal split takes place down the middle of this double row, an equal division of the nucleus between the two daughter individuals being thus effected. In the daughter individuals the nuclear material passes through the above series of stages in exactly reverse order, until the spiral band characteristic of the adult Trypanosome is reconstituted.

---

* Schaudinn, "Generations- und Wirtswechsel bei Trypanosoma und Spirochaete" (Vorl Mitteil), 'Arbeit a. d. Kaiserlichen Gesundheitsamt,' vol. 20, 1904.

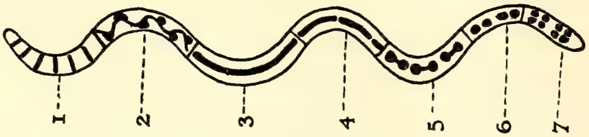


Fig. 2.—Diagrammatic representation of the nuclear changes occurring in longitudinal division. 1. Nuclear band as in normal Trypanosome, form more ladder-like than spiral. 2. Spiral thread with chromatin masses. 3. Nuclear band condensed to longitudinal rod. 4. Rod segmented. 5. Stage with dumb-bell shaped segments. 6. Chromosomes. 7. Chromosomes divided into two in the longitudinal plane of the animal. The grouping into fours is due to the fact that the pair of chromosomes derived from one dumb-bell shaped segment remain close together.

Formation of the Gametes.—The gametes are developed in the crystalline style of the oyster, their production being apparently occasioned by the melting of the style itself; as large numbers of gametes, large in the case of the male at any rate, are obtained, when, by keeping the oyster out of water, the style is caused to disappear.

The development of the male gamete alone has been observed, but it seems probable that that of the female runs much the same course.

The male gamete is produced from the indifferent form by one longitudinal division (fig. 3), nuclear substance being extruded from the Trypanosome near its middle during the division. One indifferent form thus gives rise to two gametes, the longitudinal division corresponding to the equation division, the extrusion of nuclear material to the reduction division, of higher forms.

The nuclear band undergoes the same changes as in preparing for an ordinary longitudinal division, with the exception that, at the stage when the nucleus is in the condition of a series of dumb-bell shaped segments,

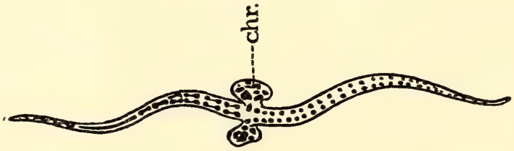


Fig. 3.—Diagram representing some of the stages in the process of nuclear change occurring in the formation of the male gamete. chr., chromatin extruded at centre. The upper part of the Trypanosome shows the nuclear band in various stages of change from spiral thread to double row of dumb-bell shaped segments. The lower half shows the double row of chromosomes.

these, instead of dividing transversely to form two chromosomes, divide longitudinally, producing two longitudinal rows of dumb-bell shaped masses.

Each of the masses now divides transversely, making a double row of chromosomes. The number of the chromosomes is now reduced to one-half, half the chromosomes of each longitudinal row being extruded at the centre of the dividing Trypanosome. The number of the chromosomes in the fully developed gamete appears to be 32. In its final form the male gamete is a much elongated mass of protoplasm containing 32 spherical masses of chromatin arranged at equal intervals along its length. The periplast is exceedingly thin. An undulating membrane may be, but more often is not, present.

The gamete is very susceptible to external conditions, rapidly degenerating when these become unfavourable.

Female Gamete.—Only two cases of the earlier stages of conjugation have been seen in stained preparations, and here the female gamete is seen to be similar to the male, only of greater thickness.

Conjugation.—Conjugation appears to take place at the moment of the style's dissolution, and to be of rare occurrence. In the two presumed cases of conjugation obtained, the male is seen to be lying in close apposition to the female for about one-sixth of its length, the remainder of the two gametes being free from one another. Later stages, including the zygote, have unfortunately not been obtained.

Encystment.—This has been observed to take place in culture and within the gut of the oyster. Encystment of the indifferent and female forms is to be distinguished, nuclear changes indicative of parthenogenesis taking place in the case of the latter.

Encystment of Indifferent Forms.—The forms about to undergo encystment cease to swim irregularly about, remain in one spot and enter upon a phase of activity, in which characteristic motions, varying considerably in different cases, are performed, a periodic reversal of direction being, however, common to all. During the motile period the nuclear band undergoes the series of changes described in the case of longitudinal division, condensing and breaking up into a number of isolated chromosomes. No differentiation out of the karyosom occurs. Motion ceases and the periplast bursts, protoplasm and nuclear elements flowing out to form an irregular mass, which condenses to form a spherical cyst. The chromosomes undergo secondary fusion into irregular masses and later become indistinct. The periplast takes no part in the formation of the cyst. This method of encystment is reminiscent of the spore formation of the bacteria. The cysts do not appear to have a well developed membrane, although the contour is sharply defined. The absence of a hard shell or tough membrane is probably correlated with the aquatic environment of the oyster, which would render only slight protection of the cyst contents necessary.

Encystment of the Female Forms.—In all its general features this resembles that of the indifferent forms, the cysts alone being larger in correspondence with the larger size of the females themselves. In point of nuclear change, however, as above mentioned, important differences manifest themselves. When encystment is comparatively far advanced, and the majority of the cell contents have emerged from the periplast, two small masses of deeply staining nuclear substance, connected by a feebly staining thread, are to be observed in favourably stained specimens.

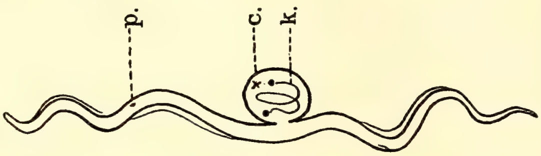


Fig. 4.—Schematic representation of female form encysting. p., empty periplast. c., cyst in process of formation. k., karyosom.

This thread, with its two terminal chromatin masses, I interpret as being the karyosom of the nuclear band, with its chromatin aggregated at each end. This karyosom may occupy various positions with regard to the rest of the nucleus, which is now condensed, but usually passes through it. The two terminal masses of chromatin now appear to divide two or more times, the products of division remaining always connected to one another by a colourless thread. Most of these daughter bodies degenerate, but two remain over, one at each end of the thread, and appear to conjugate with one another. The whole process recalls somewhat the nuclear changes of the parthenogenesis described by Schaudinn in the case of *Trypanosoma noctuæ.* The fate of the karyosom after the conjugation, and of the main portion of the nucleus is difficult to trace, but both structures become indistinct and possibly diffused throughout the cyst protoplasm, giving rise to a chromidial condition similar to that described by R. Hertwig in the case of *Actinosphærium.*

Summary of the Life History.

In summarising the main points in the life history of *Trypanosoma balbianii* it is seen that the whole of the developmental cycle with the exception of the growth of fresh individuals from the cysts, takes place in the gut of a single host. In the style the Trypanosomes undergo longitudinal division as the normal method of multiplication, and when the number has become considerable, in many cases encyst at the periphery of the style. In the style also many individuals undergo degeneration, the empty shells of periplast and torn off undulating membrane occurring at times in considerable quantities. When the style disappears production of the gametes occurs, followed by conjugation. When for any reason the oyster is subjected to a hunger period and the style disappears, the indifferent forms either encyst or multiply rapidly, becoming much attenuated in the process. The female forms are, however, more resistent, and under these conditions do not multiply, but after a time encyst. Intra-epithelial stages in the gut do not appear to occur.

Transmission of the parasite appears to take place by cysts alone.

The points of greatest interest brought to light in the study of *Trypanosoma balbianii* are perhaps the following :—

(1) The primitiveness of the nuclear relations in the normal individual.

(2) The extraordinary variability of the undulating membrane, a variability which has not been correlated with any definite conditions of life.

(3) The method of nuclear change, involving the condensation of the spiral nuclear band into a rod which segments into chromosomes.

(4) The method of encystment and condition of the nucleus recalls the bacteria.

(5) The existence of appearances in the encystment of the female forms suggestive of parthenogenesis.

(6) The method of the formation of the male gametes, involving a longitudinal division and the extrusion of nuclear substance.

(7) The resistant nature of the female forms and great susceptibility of the male gametes to unfavourable conditions.

(8) The correlation of the production of gametes and occurrence of conjugation with the disappearance of the style.

On the Chemical Mechanism of Gastric Secretion.

By J. S. Edkins, M.A., M.B. Cantab., Lecturer on Physiology in the Medical School of St. Bartholomew's Hospital, London.

(Communicated by Professor C. S. Sherrington, F.R.S. Received May 13,—Read May 18, 1905.)

It has long been known that the introduction of certain substances into the stomach provoke a secretion of gastric juice. This is regarded as in no sense depending upon mere mechanical stimulation of the mucous membrane, and it has been thought that the nervous mechanism of the gastric glands may be susceptible to certain local chemical stimuli.

On the analogy of what has been held to be the mechanism at work in the secretion of pancreatic juice by Bayliss and Starling, it is probable that, in the process of absorption of digested food in the stomach, a substance may be separated from the cells of the mucous membrane which, passing into the blood or lymph, later stimulates the secretory cells of the stomach to functional activity. The following observations support this view:—

If an extract in 5 per cent. dextrin of the fundus mucous membrane be injected into the jugular vein, there is no evidence of secretion of gastric juice. If the extract be made with the pyloric mucous membrane, there is evidence of a small quantity of secretion. With dextrin by itself there is no secretion.

Extracts of fundus mucous membrane in dextrose or maltose give no secretion; extracts of pyloric mucous membrane give marked secretion; dextrose or maltose alone bring about no secretion.

If extracts be made with commercial peptone, it is found that no secretion occurs with the fundus mucous membrane, a marked secretion with the pyloric mucous membrane; the peptone alone gives a slight secretion.

If the extracts be made by boiling the mucous membrane in the different media, the effect is just the same, that is to say, the active principle, which may be called "gastrin," is not destroyed by boiling.

Finally, it may be pointed out that such absorption as occurs in the stomach apparently takes place in the pyloric end. With the pig's stomach, in which the true cardiac region differs from the typical fundus region in having only simple glands as in the pyloric, extracts of the cardiac region in general have the same efficacy in promoting secretion, as do pyloric.

A Preliminary Note on the Susceptibility of Goats to Malta Fever.

By Dr. T. Zammit, Member of the Mediterranean Fever Commission.

(Communicated by Colonel D. Bruce, C.B., F.R.S., R.A.M.C. Received July 25, 1905.)

Experiment 1.—White Goat. To note the effect of feeding goats on material containing *Micrococcus melitensis*—

1904, Sept. 15.—Examined blood for agglutination. Negative.
18.—Fed this goat, adding the contents of an agar tube culture of *M. melitensis.*
Dec. 3.—Blood has reacted in dilutions of 1 in 20 to 1 in 100, but the temperature curve shows no rise. Fed again in the same way.
23.—Blood reacts 1 in 300.
1905, Apr. 29.—Blood reacts 1 in 100. Goat still alive.

Experiment 2.—Red Goat—

1904, Dec. 3.—Blood reaction negative. Fed as in Experiment 1.
5.—Fed again.
15.—Blood reaction negative.
23.—Blood reacts 1 in 20; 1 in 50 after half an hour.
1905, Apr. 29.—Blood reacts 1 in 50.

These two experiments led me to the belief that goats are susceptible to Malta fever, and that the disease may be spread to human beings by goats.

Experiment 3.—Examination of the blood of goats suffering from naturally acquired Malta Fever by the agglutination test—

1905, June 14.—Examined the blood of six goats which were bought out of two different herds on the 12th inst.

Goat No. 1.—Strong immediate reaction, in dilution of 1 to 20.
Goat No. 2.—Strong immediate reaction, in dilution of 1 to 20.
Goat No. 3.—Strong reaction, after half an hour.
Goat No. 4.—No reaction.
Goat No. 5.—Strong reaction, after half an hour.
Goat No. 6.—Strong immediate reaction.

On June 15 the bloods were again examined, with identical results. On

June 18 about 5 c.c. of blood was taken from Goat No. 6 and distributed in six broth-tubes. On June 25 passages from the broth-tubes were made on to agar slopes, and the *M. melitensis* recovered in pure culture. This micro-organism was also recovered from the blood of Goat No. 5. Blood was also taken from Goats Nos. 1, 2, and 3, but so far the *M. melitensis* has not been recovered.

Material from Abattoir.—Dr. Caruana Scicluna having suggested that possibly infected goats might be met with in the abattoir, I have examined 46 spleens, and have recovered the *M. melitensis* from one. The blood of seven of these goats gave a positive reaction to the agglutination test.

Preliminary Note on Goats as a Means of Propagation of Mediterranean Fever.

By Major W. H. Horrocks, R.A.M.C., Member of the Mediterranean Fever Commission.

(Communicated by Colonel Bruce, C.B., F.R.S.—Received July, 1905.)

With the object of ascertaining, by experimental inoculation, whether goats could be infected by the *M. melitensis,* six goats were bought on June 12, 1905, from two different herds, and placed in the lazaretto. On June 14 Dr. Zammit, as a preliminary step to our experimental work, took blood from each of these goats, and proceeded to test the action of the serum on the *M. melitensis.* He found, to his great surprise, that the serum of five of the goats, when considerably diluted, caused agglutination of this microbe. On June 15 similar results being again obtained, Dr. Zammit brought specimens of the bloods to the Public Health Laboratory, and asked me to confirm his observations. I obtained the following results:—

Goat No. 1.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis,* visible to the naked eye. When diluted 1 to 100, however, the serum gave no reaction.

Goat No. 2.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis.* A dilution of 1 to 100 produced a complete reaction after 15 minutes.

Goat No. 3.—Blood serum diluted 1 to 10, 1 to 40, and 1 to 100, caused immediate agglutination of the *M. melitensis*, but, in the case of the dilution 1 to 100, the clumps were not visible to the naked eye until after 15 minutes.

Goat No. 4.—The blood serum produced no reaction with the *M. melitensis.*

Goat No. 5.—The blood serum diluted 1 to 10 caused immediate agglutination, but dilutions of 1 to 40 and 1 to 100 did not produce a complete reaction until after 15 minutes.

Goat No. 6.—Blood serum diluted 1 to 300 caused complete agglutination of the *M. melitensis*, visible at once with the naked eye.

The reactions thus obtained, and especially that of Goat No. 6, suggested that possibly five of the goats were suffering from Mediterranean Fever acquired under natural conditions. The goats were stated to be healthy, but were sold cheaply, as they had given very little milk for some time. They were bought from pens in the neighbourhood of Birchircara and St. Julians, and taken straight to the lazaretto, where they were placed in clean stalls, which had never been used for any experimental work with the *M. melitensis.*

Dr. Zammit and I then arranged to make a complete study of these animals; Dr. Zammit undertook the investigation of the blood, and I made myself responsible for the bacteriological examination of the milk and urine.

Bacteriological Examination of Milk and Urine obtained from Naturally Infected Goats.

Goat No. 6.—I commenced work with this goat, as its blood serum, when diluted 1 to 300, caused immediate agglutination of the *M. melitensis.* The animal did not appear well, and had a very poor coat. The udders were flaccid, but the milk exuded appeared normal in character. The temperature was taken morning and evening, and compared with that of a healthy goat. The evening temperature never rose above 103°, and, as this temperature is often recorded in the case of perfectly normal goats, a febrile temperature could not be said to be present. On June 18 milk was withdrawn, and 1 c.c. centrifugalised; the deposit was then carefully spread over 10 litmus-nutrose-agar plates. After four days' incubation at 37° C., colonies of the *M. melitensis* appeared in every plate. The colonies were at once tested with a dilute (1 to 100) specific serum obtained from an inoculated rabbit. The micrococci were found to agglutinate at once, the clumps being visible to the naked eye. Some of the colonies were then planted out on agar slopes, and the resulting growths, when subjected to the usual confirmatory tests, showed that the *M. melitensis* was undoubtedly being excreted in the milk of this goat.

On June 22 the milk was again examined and the *M. melitensis* recovered once more.

On June 23 examination of the urine was commenced. The vagina was washed out with an antiseptic solution and a catheter, previously sterilised in boiling water, passed into the bladder. The urine so obtained was plated on litmus-nutrose-agar, but after four days' incubation at 37° C., in spite of the precautions taken, the plates were found densely crowded with saprophytic organisms, and the *M. melitensis* could not be detected.

On June 24 and 26 the urine was again plated, the same precautions being used, but the plates were densely crowded with foreign organisms, and the *M. melitensis* could not be seen.

On June 27, 28, 29, and 30, and on July 1, 3, 4, 5, 7, 8, 9, and 10, the urine was also examined, but up to the present the *M. melitensis* has not been recovered.

The milk was plated again in June and July, and the *M. melitensis* was found on each occasion.

Result.—The *M. melitensis* appears to be steadily excreted in the apparently normal milk of this goat, but up to the present it has not been found in the urine.

Goat No. 1.—This animal appeared healthy, but the udders were flaccid, and the milk exuded had a thin serous appearance. The temperature was taken regularly, but no indications of fever were observed.

On June 22, 1 c.c. of the milk was centrifugalised and the deposit plated. After four days' incubation at 37° C., the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were obtained.

On June 26, 29, and 30 the urine was examined, but no signs of the *M. melitensis* could be discovered.

On July 1, 10 c.c. of the urine were centrifugalised and the deposit plated; four days later every plate was found studded with colonies of the specific microbe. The colonies were fished, planted on agar slopes, and the resulting growths tested in the usual manner.

Result.—The *M. melitensis* is excreted in very large numbers in the serous looking milk of this goat. It is also excreted in the urine.

Goat No. 2.—This goat appeared quite well, and the milk exuded from the udders had a normal appearance. There were no indications of fever.

On June 22, 1 c.c. was centrifugalised and the deposit plated. After four days' incubation about 30 colonies appeared in every plate. On June 24 and 26 the milk was again examined, and colonies of the *M. melitensis* were recovered on both occasions.

The urine was examined on June 23, 26, 27, 28, 29, and 30, and on July 1, 3, and 6, but the *M. melitensis* could not be detected.

Result.—The *M. melitensis* appears to be excreted in small quantity in the normal-looking milk of this goat. It has not yet been detected in the urine.

Goat No. 3.—This goat looked healthy and had no fever, but its milk was thin and serous. On June 22 the milk was examined, one loopful of the serous milk being spread over each plate. After four days' incubation all the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were again obtained.

The urine was examined on June 23, 26, 28, and 30, and on July 1, 3, 6, 8, 9, 10, 11, but no signs of the *M. melitensis* could be discovered.

Result.—The *M. melitensis* appears to be present in enormous quantities in the thin serous-looking milk of this goat, but it has not yet been found in the urine.

Goat No. 5.—This goat was in poor condition, the udders were flaccid, and the milk exuded had a thick jelly-like appearance.

On June 22 the milk was examined, one loopful of the jelly-like material being spread over each plate. After four days' incubation all the plates were covered with minute colonies of the *M. melitensis.* On June 24 and 26 the milk was again examined, and densely crowded plates were obtained as before.

On June 25 and 30 the urine was examined, but no colonies of the *M. melitensis* were detected.

On July 1 the urine was again plated, and four days later every plate was found to contain numerous transparent colonies strongly resembling those of the *M. melitensis.* Some of the colonies were fished and planted out on agar slopes. The resulting growths were then subjected to the usual confirmatory tests, and the *M. melitensis* proved to be undoubtedly present.

The five goats just examined being considered by their owners to be "out of milk," would not be likely to be employed for milking purposes, though in the case of Goats Nos. 2 and 6, the milk might easily have been used without any fear of suspicion arising as to its being abnormal. Consequently it appeared very desirable to examine the herds which were actually supplying milk to Valetta, Sliema, and the various hospitals.

I therefore asked Captain Kennedy, R.A.M.C., to visit the various herds, and, with the owner's consent, take blood from the ears, and test the action of the sera on the *M. melitensis.* The results he obtained are given in Part VIII; it will be seen that, out of 161 goats examined, 84 gave a reaction, corresponding to a percentage of 52 probably infected with Mediterranean Fever. I then obtained samples of milk from some of the apparently infected animals, and proceeded to plate them on litmus-nutrose-agar. The following results have been obtained up to the present time:—

Examination of the Goats supplying Milk to Forrest Hospital.

I visited this herd, which assembles outside the hospital gate every morning, and selected Goats Nos. 38, 48, 37, and 43 from Captain Kennedy's list.

Goat No. 38.—The milk from this animal was centrifugalised, and the deposit plated on July 4, 5, 6, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Goat No. 48.—The milk was examined on the same dates as Goat No. 38, but, so far, the *M. melitensis* has not been isolated.

Goat No. 37.—The milk of this animal was taken on July 4, and 2 c.c. centrifugalised; the deposit was then plated. After four days' incubation every plate was found densely crowded with small colonies of the *M. melitensis;* the colonies were so numerous that it was impossible to make an accurate count. The colonies were fished and planted on agar, the growths resulting responded to all the tests characteristic of the *M. melitensis.*

On July 5 and 6 the milk was again plated, and similar results were obtained.

As this goat was in full milk, there cannot be any doubt that the *M. melitensis* was being excreted in large numbers. A pint of the milk was then collected, and Dr. Zammit very kindly made a chemical examination of the sample. The result given below shows that the milk was of good quality.

Analysis of Milk from Goat No. 37.

Density at 15° C.	1030	
Fat	4·3	per cent.
Total solids...........................	13·18	„
Solids, non-fat	8·8	„
Ash....................................	0·51	„

Goat No. 43.—The milk of this goat was examined on July 4, 5, 6, 7, 8, 9, and 10, but, up to the present, the *M. melitensis* has not been isolated.

A reference to Captain Kennedy's list shows that, while Goat No. 37 reacted in a dilution of 1 to 60, Goats Nos. 38, 48, and 43 only reacted in a dilution of 1 to 20, and were probably in an early stage of the disease.

Examination of a Small Herd supplying Milk to Valetta Station Hospital.

Goats Nos. 27, 30, and 32 were selected from this herd. The goats were kept at Casal Curmi, and brought every morning to the Station Hospital.

Goat No. 30.—On June 29 and 30 milk was centrifugalised and plated in the usual manner, but the *M. melitensis* was not detected.

On July 1 plates were again made, and a few typical colonies appeared.

On July 3 10 c.c. of the milk were centrifugalised, and the deposit plated; four days later every plate was found densely crowded with colonies of the *M. melitensis.*

On July 6 similar results were obtained.

A sample of the milk was then analysed by Dr. Zammit, and found to have an average chemical composition.

Goats Nos. 27 *and* 32.—The milk from these goats was examined on June 29 and 30, and on July 1, 3, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Examination of a Small Herd Supplying Milk to Valetta.

This herd assembled in St. John's ditch, and 17 out of 25 animals showed a blood reaction with the *M. melitensis,* and six of them reacted when the serum was diluted 1 to 100. Goats Nos. 50 and 52 were selected from Captain Kennedy's list.

Goat No. 50.—On July 6, 1 c.c. of the milk was centrifugalised and the deposit spread over the usual plates. Four days later all the plates were found densely crowded with small colonies of *M. melitensis.*

The confirmatory tests were applied in the usual manner. This animal was considered one of the best milkers in the herd, and its owner valued it at £5, whereas the ordinary price for a goat in milk varies from £3 to £4.

Goat No. 52.—This animal appeared in good health and its udders were full of milk. It was purchased and placed in the lazaretto.

On July 5 milk was withdrawn and 1 c.c. centrifugalised; the deposit was then spread over nutrose-agar plates in the usual manner. After four days' incubation at 37° C., all the plates were found so crowded with colonies of *M. melitensis* that a reliable count could not be made.

On July 6 and 8 the milk was again examined and similar results were obtained.

A sample of the milk was submitted to Dr. Zammit for chemical analysis; he obtained the following results:—

Specific gravity at 15° C................ 1031

Total solids, 14·0 per cent.; fat, 3·6 per cent.; ash, 0·73 per cent.

Examination of a Herd Supplying Milk to Sliema.

Two goats were bought from this herd and placed in the lazaretto. The pens were in the neighbourhood of Misida.

Goat No. 15.—On July 5 the blood was examined and the serum, diluted 1 to 50, was found to cause complete agglutination of the *M. melitensis* visible to the naked eye. The goat appeared to be in good health, and the udders were full of milk. Some milk was withdrawn and 2 c.c. centrifugalised; the deposit was then plated in the usual manner. On July 9 the plates were found covered with small colonies of the *M. melitensis.*

On July 6 the milk was again examined, and the deposit from 1 c.c. produced as before an immense number of colonies of *M. melitensis.*

The urine was withdrawn by a catheter and plated on July 5, 6, 7, 8, 9, and 10, but up to the present the *M. melitensis* has not been isolated.

A chemical analysis of the milk was made by Dr. Micalef, with the following results:—

Total solids, 13·5 per cent.; fat, 4·1 per cent.; ash, 0·75 per cent.

Goat No. 16.—This goat was taken from the same herd as No. 15. On July 4 the blood was examined, and the serum diluted 1 to 60, was found to cause immediate clumping of the *M. melitensis.* The milk and urine have been examined daily since July 4, but up to the present the *M. melitensis* has not been isolated from either source.

Conclusions.—The results obtained show that some of the goats in every herd examined are suffering from Mediterranean Fever. The *M. melitensis* is exuded in the milk in enormous numbers when the disease has been present sufficiently long to cause a change in the physical characters of the fluid. It is also excreted in considerable numbers even when the animals are in "full milk," and no changes have occurred in either the physical or chemical characters of the milk.

The *M. melitensis* is also excreted in the urine of goats suffering from Mediterranean Fever, but up to the present it has only been found when the disease has existed for some time and physical changes have occurred in the milk.

On the Occurrence of Certain Ciliated Infusoria within the Eggs of a Rotifer, considered from the Point of View of Heterogenesis.

By H. Charlton Bastian, M.A., M.D., F.R.S.

(Received February 14,—Read March 16, 1905.)

(Plate 7.)

(Abstract.)

The weight of preconceptions against the possibility of the occurrence of Heterogenesis has hitherto been so strong as to have made it almost impossible to obtain any adequate consideration for the actual evidence adduced in favour of this or that alleged instance. But of late, preconceptions in the domain of physics and chemistry have received severe shocks, and when we are told that a so-called "element" is daily being transformed and another is actually originating therefrom, there appears more chance of attention being paid to the alleged existence of phenomena in the organic world which would seem to be but the carrying on into a higher platform of the familiar but important phenomena known as allotropism and isomerism.

Hitherto, alleged instances of heterogenesis have, without adequate consideration of evidence, been almost always assumed to be results of "infection," but the writer claims that in the cases with which the present article is concerned, any such explanation is quite impossible in regard to one of the cases, at least, in which we have masses of living matter so large that they average $\frac{1}{8}$ mm. in diameter, being converted in the course of three days into great Ciliated Infusoria of equal bulk.

The communication deals with two sets of heterogenetic transformations occurring in the great eggs or "gemmæ" of one of the largest of the Rotifers, namely, (1) the transformation of the entire contents of a Hydatina egg into a single great Otostoma; and (2) the segmentation of the Hydatina egg into 12 to 20 spherical masses, and the development of these sometimes into embryo Vorticellæ, and sometimes into embryo Oxytrichæ.

Rotifers and Ciliated Infusoria belong to such distinct phyla of the animal kingdom that no immediate kinship can possibly obtain between them, and consequently heredity, in the ordinary sense of the term, can have nothing to do

with the forms assumed by the Ciliates taking origin from such a source. Thus, if I can prove that two or more wholly different forms of Ciliated Infusoria can be produced from transformations of the substance of the eggs of a particular Rotifer, I shall not only establish the reality of heterogenesis in one of its most striking phases, but shall go far to show that the varied forms even of such organisms as the Ciliata are as much the outcome of their molecular composition and of the laws of "polarity" as are the various forms assumed by crystalline matter.

(1) *The transformation of the entire contents of a Hydatina egg into a great Otostoma.*—Having witnessed on very many occasions the stages of this remarkable transformation of the contents of a Rotifer's egg into a Ciliated Infusorium, I am desirous of acquainting the Royal Society with the simple procedure needful to enable zoologists to study for themselves the series of changes leading to a result which many of them may be disposed to deem incredible.

All that is necessary is to procure a good stock of these large Rotifers by placing some surface mud, having a coating of Euglenæ, from a ditch in which Hydatinæ are known to exist, into a glass bowl, and to pour thereon water to a depth of about 4 inches. In the course of two or three days (with a temperature of 16° or 17° C.), if the Hydatinæ are abundant, a good crop of their large eggs will be seen at the surface of the fluid, where it is in contact with the glass. The difficulty is to find suitable sites in which the Hydatinæ are really abundant. An excellent source from which I formerly obtained supplies has since been destroyed.

By the aid of a scalpel passed along their track for a short distance, groups of 20 to 30 eggs may be taken up at one time, and gently pressed off the edge of the blade into a small, white stone pot full of water. Some of such small masses of eggs (mixed, perhaps, with a few Euglenæ) will float, and others will sink. After seven or eight of these masses have been gathered and deposited, the cover should be placed upon the pot so as to cut off from the eggs all light rays, both visible and invisible.*

If the supply of eggs will admit of it, two other pots should be similarly charged; but if there are not enough eggs for this purpose, the two other pots should be charged on successive days with fresh batches of eggs. The larger the supplies of new-laid Hydatina eggs the more convincing will be the result. Thus on one occasion when my supply was very abundant, gatherings of eggs were made at intervals of six hours, and among such eggs as were subsequently taken from the pots I found that *from* 12 *to* 25 *per cent. yielded Otostomata rather than Hydatinæ.*

---

* Full details as to the best means of obtaining supplies of Hydatinæ, dealing with them, and subsequently obtaining the largest proportion of freshly laid eggs, will be found in my "Studies in Heterogenesis," pp. 125 and 286.

When the pots have remained covered and undisturbed for 36 hours at a temperature of about 17° C., one of them may be opened, and some of the small masses of eggs from the bottom of the pot should be taken up with a tiny pipette and placed in a drop of water on a microscope slip. Before covering the specimens, a minute fragment of a cover-glass should be placed at each side of the drop of water, so as to protect the delicate eggs from undue pressure.

On examination by a low power of the microscope it will be seen that there are many empty egg-cases, that within some eggs there are embryo Hydatinæ in different stages of development, while within the remaining eggs the contents will be wholly different, consisting of an aggregate of minute pellucid vesicles, each containing a few granules, together with a variable amount of granules interspersed among the vesicles, as in Plate 7, fig. 4.

A newly laid egg is shown in fig. 1, while figs. 3 and 4 represent two intermediate stages between it and the condition represented in fig. 4. In fig. 5 we have six Hydatina eggs and an empty egg-case, shown under a lower power, all of which were taken from one of the pots after 36 hours. The three central eggs are in the vesicular stage of transformation into Ciliates, while the three others show Hydatinæ in different stages of development.

If the cover should be again placed upon this pot with a view to the examination of other portions of its contents 24 or 36 hours later, and this examination is made, it will be found that no further advance has taken place, that the eggs previously in the vesicular condition still remain in this stage, and are, in fact, no further developed than some of their fellows were when previously examined.

I have found on many occasions that the opening of a pot at an early stage of the transformation—even for only four or five minutes—arrests the whole process of change. It was for this reason that I advised three separate pots to be charged, so that their contents might be examined at different periods.

When, however, a second pot is opened two and a-half or three days after the eggs have been placed therein, and portions of its contents are examined in the same way, a larger proportion of empty egg-cases will be seen. There may be few or even no developing Rotifers still remaining within the eggs, and in other egg-cases, instead of the motionless vesicular contents previously seen, great Ciliates may be found slowly revolving within the egg-case, as with the specimen shown in fig. 6 before it was killed by a weak iodine solution; or else, under the influence of the light, rupturing the egg-case, struggling out, as in fig. 7, and swimming away with rapid movements, partly of rotation. Some of the Infusoria before they emerge undergo segmentation into two, four, or rarely, even into eight smaller Ciliates. An undivided specimen which had been swimming about for half an hour, and in which the cilia are better developed, is shown in fig. 8.

The large undivided Infusoria have their bodies densely packed with large corpuscles (modified representatives of the vesicles of an earlier stage); and a large elongated nucleus which can be seen in some of them, though generally not well without the aid of reagents. They possess the characteristic ear-shaped mouth indicated by the name Otostoma, and cilia are distributed all over the body in longitudinal lines, so as to give the appearance of a delicate longitudinal striation—both these features being visible in the starved specimen represented in fig. 9.

In the event of the Ciliates being not yet fully developed at the time that the second pot is opened, we have the third pot, whose contents we can investigate slightly later.

As a control experiment it will be well at the time that the pots are charged to place two or three batches of the eggs with some of the same water into a watch glass, which is left exposed to light; and at the expiration of three or four days, as well as at later periods, to search among its contents for any of the same large Ciliates, and also for any eggs in the intermediate vesicular stage above referred to. I have invariably found that such a search yielded only negative results.

In taking batches of eggs, in the manner indicated, to be placed in the pots, individual eggs will necessarily be of different ages. Some will have already begun to develop into Rotifers, and some of these, under the altogether unnatural conditions to which they are subjected in the dark pots, are apt to become more or less malformed as development proceeds. Others that have been quite recently laid will not have begun to develop, and it is these latter eggs apparently, which, under the cutting off not only of ordinary light but probably of some invisible light rays, become speedily transformed into great Ciliated Infusoria. Cutting off ordinary light rays alone from the eggs, by placing them in a small covered glass dish shut up in a cupboard or box and maintained at the same temperature as before, seemed at first not to lead to similar results; but I subsequently ascertained that the transformation will occur under such conditions, though only after the lapse of about nine days. It looks, therefore, as if the stoppage of some invisible rays, capable of passing through wood but not through stone, notably hastens the process.

Briefly enumerated, the stages of the transformation are these:—

(1) A freshly laid Hydatina egg; (2) partial rearrangement of its contents on the way to the formation of a mass of small vesicles; (3) the conversion of the major part of the egg substance into a mass of spherical vesicles of varying sizes; (4) further changes in the aggregate of vesicles and intervening granules; (5) the formation of the embryo Otostoma within an almost invisible hyaline endocyst; (6) the development of cilia and the slow rotation of the embryo within this envelope; (7) the bursting of the hyaline envelope, with freer play of cilia and more rapid movements within the egg-case; (8) in some instances fission into two, four, or more, active segments; (9) rupture of the Rotifer egg-case, and the appearance of its contents in the form of a very large and rapidly moving Ciliated Infusorium belonging to the genus Otostoma.

During a period of eight or nine months in which I was working at this subject, off and on, I took some hundreds of the Otostomata in different stages of development from the experimental vessels. On one occasion I found two eggs within a dead Hydatina in a vesicular condition like that shown in figs. 3 and 4; and on another occasion I found a living Otostoma within a dead Hydatina, as an outcome probably from such an egg. Details as to the conditions under which they were met with are given in my "Studies in Heterogenesis," pp. 133 to 136; and in the same work full particulars will be found of the transformation of the eggs of another Rotifer (belonging to the genus Callidina) within the dead bodies of the parents into a different kind of Ciliate, belonging to the genus Glaucoma—showing that the origin of Otostoma in the manner indicated in this paper is far from being an isolated occurrence.

In the months that these observations were being made, and previously, during prolonged work with other materials taken from the same site, no Otostomata had ever been seen in association with Hydatinæ, except those that had been taken from the experimental vessels. On two occasions since, however, though from wholly different localities, Otostomata have been found pretty abundantly in association with Hydatinæ.* The adult forms have been found to be much larger, having from two to three times the length of the great embryos which issue from the egg-cases; and also to be more highly organised, seeing that what appeared to be a simple contractile vesicle has only once been seen in one of these embryo forms, while it is always present in a developed form, in association with 10 to 12 spindle-shaped radiating channels, in the adult specimens. A starved specimen of one of these Otostomata, leading a free life, showing the small ear-like mouth and the large nucleus, is shown in fig. 10.

---

* It is, of course, quite possible that on certain occasions freshly-laid Hydatina eggs in ditches may be exposed to much the same kind of conditions as those to which they would be exposed in my experimental vessels. That such a transformation does not occur oftener depends probably upon the fact that it can only be brought about in eggs which have been quite freshly laid.

Many of these adult specimens I have been able to keep for two months, and have seen them pass into an encysted condition. When in this state their bulk may be seen to be several times greater than that of Hydatina eggs. They are, likewise, enclosed in thick cyst walls, wholly unlike the thin egg-cases of the Hydatina. A rather small specimen in this condition is shown in fig. 10.

A Hydatina egg could not possibly be confounded with an adult encysted Otostoma; and the embryo Otostoma which emerges from the egg-case embodies the whole of the transformed substance of the egg. *No minute Otostoma is ever to be seen within an egg, devouring its contents.* No Ciliate is seen till the total contents of the egg having been transformed, the whole mass begins to revolve within the egg-case as a great embryo Otostoma.

(2) *The Origin of* 12 *to* 20 *Vorticellæ or Oxytrichæ from the substance of a single Hydatina Egg.*—These are most remarkable variations, which at different times have been occasionally met with in Hydatina eggs taken from the experimental vessels. Both of these changes have only been met with in eggs found at the surface of the fluid in the experimental vessels, while the transformation of the egg-mass into an Otostoma seems to occur more abundantly, away from the air, in eggs which drop to the bottom of the pots. The changes into Oxytrichæ have been met with in the spring on three separate occasions; those into Vorticellæ only once, and that in the autumn.*

The first occasion on which the transformation of the Hydatina egg-substance into Oxytrichæ was met with was in 1872, and is thus referred to in "The Beginnings of Life"†:—"The substance of some of the large thin-walled 'eggs' of *Hydatina senta* was seen to have undergone segmentation into about 16 spheres, each 1/1000″ in diameter. The external layers of these soon became condensed into cyst-walls, whilst the internal substance of each of them, after undergoing a series of molecular changes, resolved itself into an embryo *Oxytricha*, some of which might be seen revolving within their cysts. Some of this batch of Rotifers' 'eggs' were seen to be filled with such spherical masses, whilst others were observed in which a few of the embryos had escaped from their cysts, and were swimming about as well-marked specimens of *Oxytricha* within the thin investing membrane of the Rotifer egg."

---

* It is admitted that metamorphosis from one to another form occasionally occurs among the Ciliata during their stage of encystment (Carpenter on 'The Microscope,' Eighth Edition, 1901, by Dallinger, p. 780); and among such instances of alleged transformation it is of interest to note that the origin of Oxytrichæ from encysted Vorticellæ was described and illustrated by Pineau in the 'Annales des Sciences Naturelles,' 1848 (Zool.), p. 99.—*Note added June* 29, 1905.

† Vol. 2, p. 489.

Unfortunately, nothing was stated as to the conditions to which these eggs had been subjected, and it was my quest in this direction, resumed about five years ago, that finally led to the discovery of the origin of Otostomata and of Vorticellæ, as well as to the repetition of my observations concerning the heterogenetic origin of Oxytrichæ, from Hydatina eggs.

Fig. 12 shows the conversion of the egg-substance into a number of large but unequal spherical masses; while in fig. 13 another Hydatina egg is shown, surrounded by Euglenæ and Diatoms in a later stage of development. The 20 unequal spheres are exactly similar to others which were seen in contiguous eggs, whose development was traced into embryo Vorticellæ such as are represented in fig. 14.

If the egg-substance is found to have segmented into 12 to 20 more or less equal spherical masses, there is at first no means of knowing whether such masses are to be developed into embryo Vorticellæ, or into embryo Oxytrichæ. But if either of the masses is seen to be revolving within its own delicate cyst, we may be sure that this particular egg will not yield Vorticellæ, as these embryos do not revolve before rupturing their cysts, and the Hydatina egg produces either the one or the other form—never a mixture of the two.

It cannot be supposed that 12 to 20 of either of these Ciliates in an embryo condition could penetrate the egg-case, could devour its contents without being seen, and would then, as embryos, encyst themselves (all in two days, or less)—only, almost immediately after, again to pass out of their encysted condition, and to appear as the active young Vorticellæ, or Oxytrichæ, whose development I have traced.

In the normal development of a Hydatina egg it never goes through changes in which it is converted into an aggregate of minute vesicles, or into a smaller number of separate and larger spheres, such as occurs as a prelude to the transformation of the egg-contents into Ciliated Infusoria of this or that kind.

When the facts recorded in this communication are made known to other workers in different parts of the world, some of whom may have no difficulty in finding plenty of material with which to work, and who may be skilled, as I am not, in modern methods of making sections of such minute objects as Hydatina eggs, doubtless much highly interesting information will be forthcoming of a nature to satisfy cytologists as to the histological details of the transformation in question. These minute details, however, I must leave to others; all I claim is to have established the fact itself of the heterogenetic origin of different kinds of Ciliated Infusoria from the eggs of one and the same Rotifer. The seeming utter improbability of such a fact may be taken as some measure of its enormous importance for biological science, when we consider the far from simple structure of these unicellular Ciliates, that no kinship of any sort, in the ordinary sense of the word, exists between them and Rotifers, and, further, that even totally different forms of Ciliated Infusoria are capable of being produced from the egg-substance of a Hydatina according as it becomes transformed as a whole, or only after having undergone segmentation into a varying number of small spherical masses.

DESCRIPTION OF PLATE.

(PLATE 7.)

Figs. 5 *and* 14 *are magnified* 125 *diameters, while all the others are magnified* 250 *diameters.*

FIG. 1.—A newly-laid egg, or "gemma," of Hydatina.

FIGS. 2, 3, 4.—Different stages in the transformation of the egg-substance into a *motionless* aggregate of vesicles and granules, different from anything which is ever to be seen during the normal development of the Hydatina egg.

FIG. 5.—Six Hydatina eggs and an empty egg-case, taken from one of the pots after 36 hours. The three central eggs are in the vesicular stage of transformation into Ciliates, while the three others show different stages in the development of Hydatinæ.

FIG. 6.—An embryo Otostoma which was seen *slowly revolving* within a Hydatina egg-case, taken from a pot after the expiration of three days.

FIG. 7.—An Otostoma escaping from the egg-case after the application of a weak iodine solution.

FIG. 8.—An Otostoma which was seen to rupture, and emerge from, an egg-case. Killed after about half an hour. Cilia now numerous, and well-developed.

FIG. 9.—A starved Otostoma, showing the small ear-like mouth, a very large elongated nucleus dimly indicated above and to the left, and fine longitudinal surface markings caused by rows of cilia.

FIG. 10—A small fully-developed Otostoma in a starved condition, showing small ear-like mouth and large nucleus.

FIG. 11.—A rather small Otostoma encysted.

FIG. 12.—Segmentation of a Hydatina egg into a number of large vesicular bodies.

FIG. 13.—Presumed later stage of such an egg as is shown in the last figure, now divided into about 20 unequal, evenly granular, spherical masses, such as were seen in contiguous eggs to give rise to minute embryo Vorticellæ.

FIG. 14.—Three empty egg-cases (save for one remaining sphere), with a number of the young Vorticellæ in different stages of development.

Bastian.

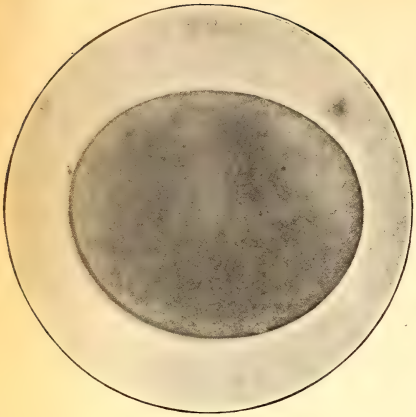


FIG. 1.

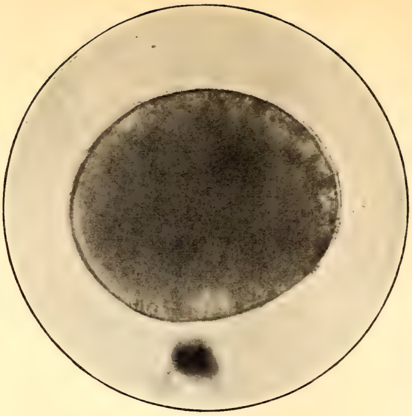


FIG. 2.

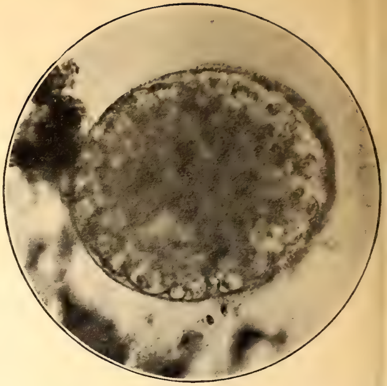


FIG. 3.

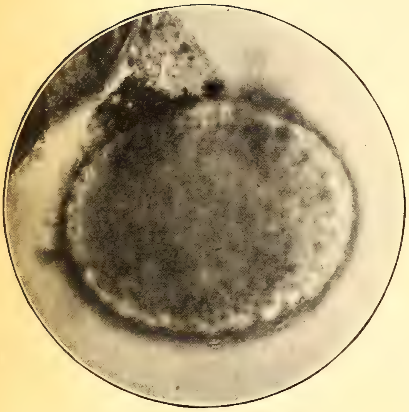


FIG. 4.

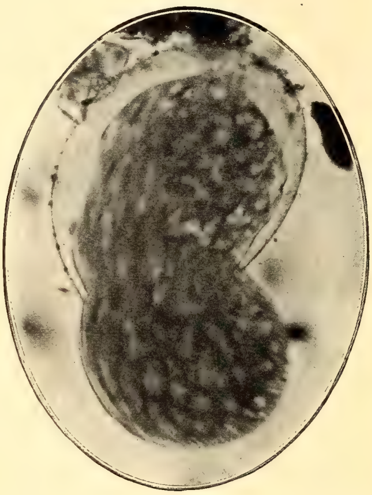


FIG. 7.

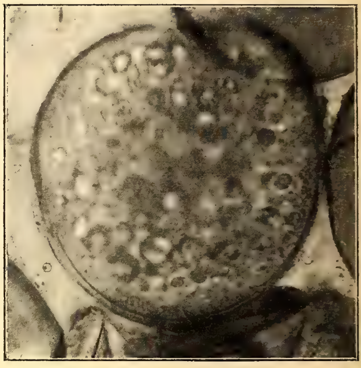


FIG. 6.

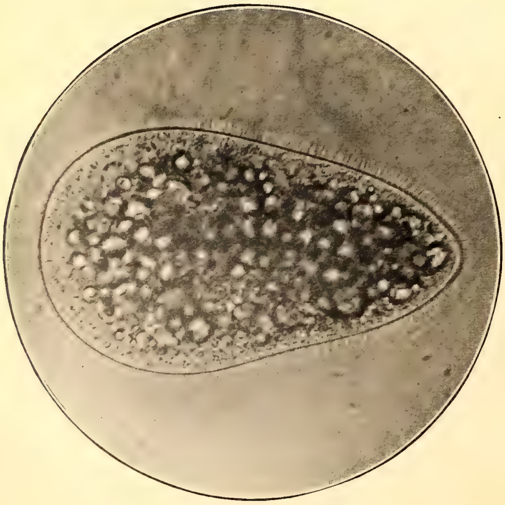


FIG. 8.

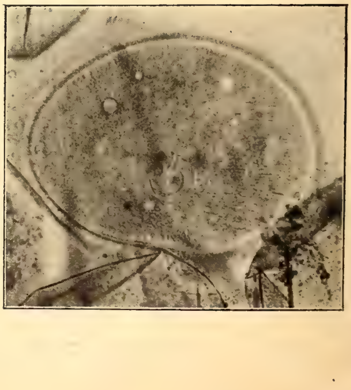


FIG. 9.

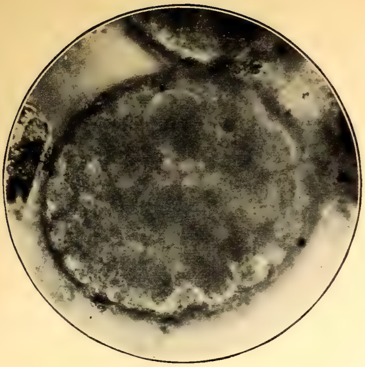


Fig. 12.

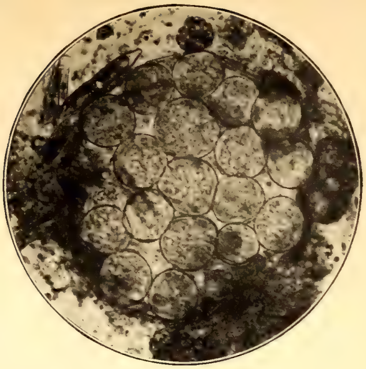


Fig. 13.

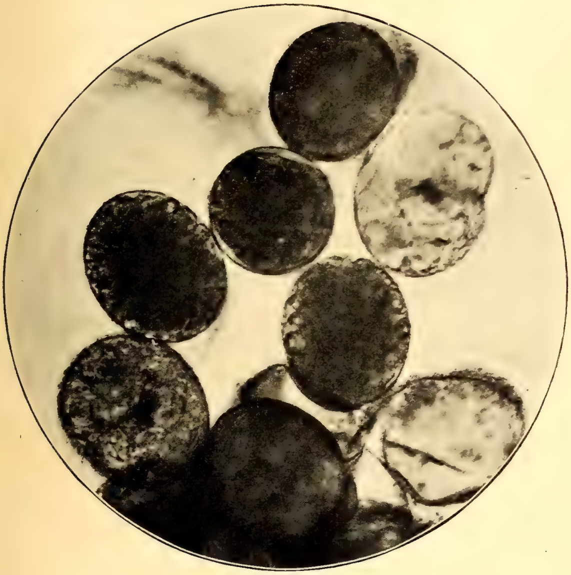


Fig. 5.

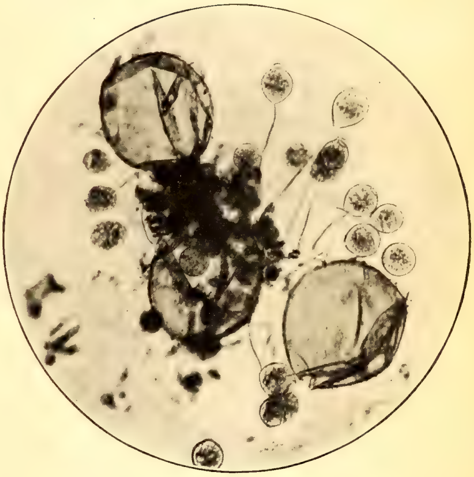


Fig. 14.

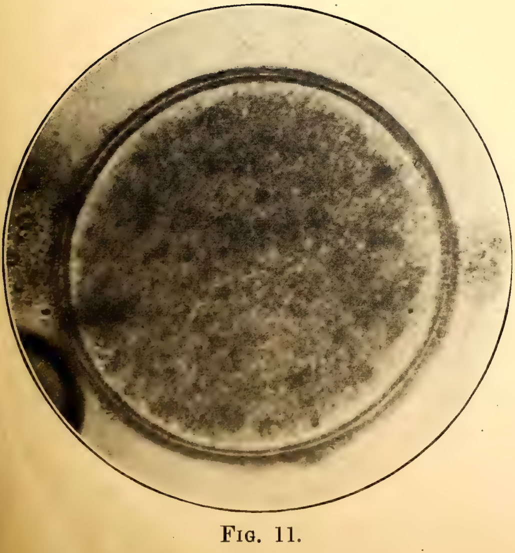


Fig. 11.

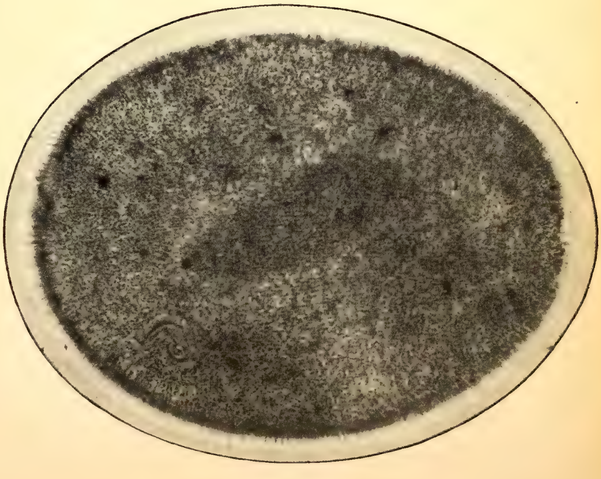


Fig. 10.

The Morphology of the Ungulate Placenta, particularly the Development of that Organ in the Sheep, and Notes upon the Placenta of the Elephant and Hyrax.

By Richard Assheton, M.A., Lecturer on Biology in the Medical School of Guy's Hospital, University of London.

(Communicated by A. Sedgwick, F.R.S. Received April 18,—Read June 8, 1905.)

(Abstract.)

The formation of the placenta of the Ungulata vera is founded on a system of foldings of the subzonal membrane (or of the trophoblast only), which fit into corresponding grooves in the walls of the uterus, without thickening of the trophoblast layer of the blastocyst, and without destruction of maternal epithelium or other tissue (*Sus*). Certain parts of the crests of the ridges are produced by local amplification into true villi, into which the splanchnopleure of the allantois subsequently extends (*Equus*, *Bos*, etc.).

For this type of placentation, which is caused fundamentally by the folding of the trophoblast, the term plicate is used (placenta plicata), and to this type of placentation it is suggested that the Cetacea, Sirenia, and Proboscidea conform, as well as the Ungulata vera, and possibly the Edentata and Prosimia.

The term placenta cumulata is used for the type of placentation in which the placenta is formed by the heaping up or thickening of the trophoblast layer, among the cells of which accumulation extravasated maternal blood circulates. Destruction of the maternal epithelium probably always occurs. To this type belong the Rodentia, Insectivora, the Hyracoidea, Primates Chiroptera. The Carnivora are perhaps intermediate, but, according to Strahl's account, they would be distinctly plicate, while, according to the account of other authors, they are slightly cumulate.

The morphological position of the Sheep's placenta, a full account of the development of which is given in the paper, is at that end of the series of plicate forms which closely approximates to the cumulate type.

Though essentially plicate in mode of development, a slight tendency to a heaping up of the trophoblast occurs, in which a distinction into a plasmoditrophoblast and a cytotrophoblast can be detected. The uterine epithelium is destroyed over certain areas in an early stage of pregnancy, and the plasmoditrophoblast forms a layer of cells, which has been mistaken for a

degenerate uterine epithelium. A direct protoplasmic connection is effected between fœtal and maternal cells.

Extravasated blood fills lacunæ between the mother and the fœtus in the cotyledonary areas, in which the shorter villi and the bases of the longer villi lie bathed in maternal blood, thus leading up to a condition characteristic of the cumulate type.

The placental connection is restricted during the later period of pregnancy to the lower part of the uterus, while the upper parts of the horns become specially active in secretion, and even exhibit a general destruction of tissue to form pabulum. At birth, a large amount of fœtal tissue is left within the uterus.

The anatomy of the Elephant's placenta at half term and at full term is shown to be closely comparable to that of the sheep. The placenta is essentially plicate, and its special peculiarities are easily derivable from those of the sheep. Its most obvious difference, the zonary as contrasted with the polycotyledonary form, is regarded as subsidiary in morphological importance to the anatomical and developmental characters. On the ground of placentation, the elephant may well be associated with the Ungulata.

Procavia (*Hyrax*), which is sometimes classified with the Sub-Ungulata, is shown, on the other hand, by its placentation to be in no way associated with the Ungulata vera, or with the Proboscidea. It is typically cumulate.

The zonary form of placentation in this case (*Hyrax*) is not to be compared with any other form of zonary placenta. The placenta of *Hyrax*, some early stages of which are described, is remarkable for the highly diffuse and cumulate placentation prior to the assumption of the zonary form.

The terms cumulate and plicate are proposed as expressing fundamental differences in the behaviour of the trophoblast which give rise to two main types of placentation and correspond on the whole to the divisions deciduate and non-deciduate, although the Carnivora which have the most deciduate of all placentas are probably to be regarded as plicate rather than cumulate.

The placentation of the Ungulata shows that that order is more closely connected with the Proboscidea, and the Sirenia, and Carnivora, than with other groups of mammals, whilst the placentation of the Hyracoidea suggests no connection at all with those groups, but is of the cumulate type, and resembles more closely the form found in certain of the Insectivora.

Contributions to the Physiology of Mammalian Reproduction. Part I.—*The Œstrous Cycle in the Dog.* Part II.—*The Ovary as an Organ of Internal Secretion.*

By Francis H. A. Marshall, Carnegie Fellow in the University of Edinburgh; and William A. Jolly, Assistant to the Professor of Physiology in the University of Edinburgh.

(Communicated by Professor E. A. Schäfer, F.R.S. Received May 13,—Read May 18, 1905.)

(Abstract.)

PART I.

The Œstrous Cycle in Carnivores.—The bitch is monœstrous and has typically two sexual seasons in the year. The wild species of the genus in their natural state have only one sexual season annually, but in captivity they may experience two sexual seasons like the bitch. The Cape hunting-dog (*Lycaon*) in captivity has been shown to come on heat usually only once a year.

The domestic cat has three or four sexual seasons in the course of the year. We find also that it is polyœstrous and may have four heat-periods in a single sexual season. The duration of the diœstrous cycle is about a fortnight. The wild cat probably experiences only one sexual season in the year. The male possesses a rutting season.

The lioness, failing pregnancy, may have several annual sexual seasons, at the same time being polyœstrous. The duration of the diœstrous cycle is said to be three weeks. Bears, polecats, and seals, and probably most other carnivores, appear to be monœstrous and breed once a year; but the otter, in captivity at any rate, is polyœstrous, having a continuous series of diœstrous cycles, each of a month's duration.

The periodicity of œstrus is dependent to some extent upon environmental conditions as illustrated in the case of the fox. Domestication and captivity appear to favour increased frequency in the recurrence of the cycle.

The Histology of the Uterus during the Cycle.—The histological changes which the uterus undergoes may be divided into the following periods:—

(1)	Period of rest	Anœstrum.
(2)	„ growth and congestion	Proœstrum.
(3)	„ destruction	
(4)	„ recuperation	Œstrus. Metœstrum.

The second period is characterised by congestion and increase in the number of capillaries. This is followed in the next period by extravasation of blood and emigration of polymorphs. External bleeding is shortly afterwards observed, having been preceded by a flow of mucus, containing polymorphs. There is considerable denudation of uterine epithelium, but the denudation does not extend to more than a single layer of stroma cells. In the recuperation period the epithelium is re-formed and new capillaries arise. This stage is also characterised by the large number of leucocytes that occur free in the stroma. These are of several kinds: (1) Large mononuclear leucocytes containing iron pigment derived from the extravasated red corpuscles, (2) coarsely granular eosinophil leucocytes, and (3) basophil cells. The latter, which occur in unusual abundance both in the stroma and in the muscle layers, are often very large. It is obvious that the changes occurring in the uterus throughout the cycle are homologous with those which are undergone in the sheep, the ferret, the monkey, and the human female.

Ovulation and the Vitality of the Spermatozoa.—Ovulation in the bitch takes place after external bleeding has been going on for several days. It occurs during the period of œstrus but it is quite independent of coition or of the presence of spermatozoa in the uterus.

No systematic investigation as to the vitality of mammalian sperms has hitherto been attempted. It has been ascertained by us, however, that the period of survival in the male passages of rabbits is probably not more than 10 days.

Some Theoretical Considerations.—The fact that ovulation does not take place until after the procœstrum (or at any rate until after the commencement of the external bleeding stage of the procœstrum) is in opposition to the view that heat and menstruation are produced by ovulation, or by the corpus luteum. It is also contrary to the theory that the degeneration stage occurs as a result of the absence of a fertilised ovum for which the preceding growth was preparing. The theory that the destruction stage of the procœstrum is of the nature of an abortion related to an ovum released at the preceding period is untenable, owing to the comparative infrequency of the heat periods (and, therefore, of the ovulation periods) in the bitch. On the other hand, the hypothesis that the entire procœstrous process is of the nature of a preparation for the lodgment of the ovum is in accordance with the facts.

PART II.

The Cause of Heat.—As pointed out by Brown-Séquard and others, the ovaries, like the testes, exercise an influence on the general metabolism of the organism throughout the reproductive period. Ovarian medication has been employed to a considerable extent for disorders associated with the female generative organs, and in the majority of cases is said to have produced beneficial results. This method of treatment, however, is in many cases purely empirical and is adopted without regard to the condition of the ovaries from which the extract is obtained.

Several investigators have experimented with ovarian grafts both in normal and abnormal positions. From some of these experiments it would appear that portions of ovarian tissue may obtain vascular connections, and produce an effect on the general metabolism comparable to that produced by ovaries in normal animals.

It has been shown by Goltz and others that the occurrence of œstrus is not due entirely to cerebral or spinal reflexes. Our experiments have demonstrated that "heat," or a transient condition resembling it, can be produced by the injection of extracts made from ovaries in a proœstrous or œstrous condition, and that when such ovaries are successfully grafted into an animal previously deprived of its ovaries, the condition produced is identical with a normal heat, and that irrespective of the situation of the graft.

The Function of the Corpus Luteum.—Of the various theories as to the function of the corpus luteum, that of Fraenkel is the only one that is supported by experimental evidence. According to this theory the corpus luteum is the only ovarian organ of internal secretion, and exerts an influence on the generative functions generally throughout the whole reproductive period of the animal's life. Among its other functions, according to this theory, it produces heat and menstruation and controls the attachment of the ovum and the formation of the placenta. This theory is only partially correct Corpora lutea are not present during the proœstrum and are therefore only functional subsequent to ovulation.

From our own experiments upon bitches and rats we draw the conclusion that the presence of luteal tissue is necessary during the first part of pregnancy, but that the corpus luteum ceases to be functional during the later stages. In these experiments we removed the ovaries from animals at various intervals after impregnation, and found that pregnancy did not continue, except in those cases in which the operation was performed in the later stages of pregnancy. Control experiments in which the ovaries were damaged or partially removed were also performed, when it was found that the animals brought forth young.

General Conclusions.—The ovary is an organ providing an internal secretion which is elaborated by the follicular epithelial cells or by the interstitial cells of the stroma. This secretion circulating in the blood induces menstruation and heat. After ovulation, which takes place during œstrus, the corpus luteum is formed and this organ provides a further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy.

Chitin in the Carapace of Pterygotus osiliensis, *from the Silurian Rocks of Oesel.*

By Otto Rosenheim, Ph.D.

(Communicated by Professor W. D. Halliburton, F.R.S. From the Physiological Laboratory, King's College, London. Received May 27,—Read June 8, 1905.)

Professor E. Ray Lankester, who has been interested in the constitution of the carapace of certain fossil Eurypterids found in Oesel in rocks of Silurian age, placed the matter in the hands of Mr. Bather, of the Geological Department, Natural History Museum.

In March last Mr. Bather asked Professor Halliburton's co-operation in the chemical investigation, stating that a preliminary examination, made by himself and Mr. G. T. Prior, led them to believe that the material retained its chitinous nature. When the small fragments of the carapace, which were all that could be spared from the duplicate specimens in the British Museum, arrived in this laboratory, Professor Halliburton placed them in my hands, and I proceeded to examine them for chitin.

Gamgee, in his 'Text-book of Physiological Chemistry,'* gives a long list of various invertebrate structures, mainly epiblastic, where chitin has been described, but the list is only approximately accurate, as Gamgee points out that, in many cases, a chitinous composition has been ascribed to a structure solely on the ground of its insolubility in caustic alkalis and dilute acids, or even in only one of these two classes of reagents. In 1884 the list was extended by Halliburton,† who showed that the cartilages occurring in Sepia and Limulus contain a small percentage of chitin; this was confirmed in the case of Sepia by Krukenberg,‡ who found the same material in the skeletal structures in that animal. It has further been found in certain fungi.§

---

* Vol. 1, p. 299.

† 'Roy. Soc. Proc.,' No. 235, 1885; 'Quart. Journ. of Micros. Science,' vol. 25, p. 173 1885.

‡ 'Ber. d. deutsch. Chem. Ges.,' vol. 18, p. 993, 1885.

§ Gilson, 'Comptes Rendus,' vol. 120; Winterstein, 'Ber. d. deutsch. Chem. Ges.,' vols. 27 and 28.

In spite of a considerable amount of work on the composition of chitin, there is still some uncertainty as to its exact chemical structure. It has, however, been definitely removed from the albuminoids with which it was formerly classed. Its most characteristic decomposition product is the amino-derivative of sugar, known as glucosamine or chitosamine. Schmiedeberg* regards chitin as an acetyl derivative of glucosamine, whereas Fränkel and Kelly† consider it to possess a more complicated composition.

Unfortunately, the amount of material I obtained from the carapace of *Pterygotus osiliensis* was too small to allow of a complete chemical investigation; it only weighed 0·0135 gramme. It consisted of brownish scales. In order to remove inorganic substances, the scales were boiled with 3 c.c. of 3-per-cent. hydrochloric acid. Carbon dioxide was evolved during this process, and the amount of material was lessened. The treatment was repeated three times, and the last traces of hydrochloric acid were removed by boiling with distilled water until the reaction was neutral to Congo paper. The residue was subjected to consecutive treatment with 2-per-cent. potassium hydroxide (to remove organic acids), water, alcohol, and ether, in order to get rid, as far as possible, of organic substances soluble in those reagents. After this treatment the substance still showed a brown colour, and an unsuccessful attempt was made to destroy the pigment with a solution of potassium permanganate. Transparent brownish scales remained.

So far, the behaviour of the material towards reagents and solvents was identical with that of chitin. In order to obtain more conclusive proof, an attempt was made to hydrolyse the substance, and identify the typical product of hydrolysis, namely, glucosamine.

A control experiment was first made with a similar small fragment of pure chitin. I was unable, however, with such a small quantity to obtain the glucosamine hydrochloride in crystalline form; but, after the treatment with hydrochloric acid, evaporation, and finally dissolving the residue in water, its presence was demonstrated by means of its reducing action on Fehling's solution.

On subjecting the brownish scales obtained from the carapace to the same treatment, it seemed at first that concentrated hydrochloric acid did not attack them. They were therefore left in contact with the concentrated acid for some weeks, and then the whole was evaporated on the water-bath. The aqueous solution of the residue showed strong reducing properties with Fehling's test, from which the formation of glucosamine may be inferred.

The conclusion I draw from my experiments is that the general behaviour of the substance towards acids and solvents is such that it is probably chitin, and this is confirmed by the fact that, after such treatment, it yielded, on hydrolysis with concentrated hydrochloric acid, a strongly reducing substance which is presumably glucosamine. The preliminary resistance noted towards the strong acid does not seem remarkable, when one considers the hardening the material must have undergone during and after fossilisation.

---

* 'Arch. f. exp. Path. u. Pharm.,' vol. 28.

† 'Monatshefte f. Chemie,' vol. 23.

On a New Species of Cephalodiscus (C. nigrescens) *from the Antarctic Ocean.*

By E. Ray Lankester, M.A., D.Sc., LL.D., F.R.S., Director of the Natural History Departments of the British Museum.

(Received and Read June 8, 1905.)

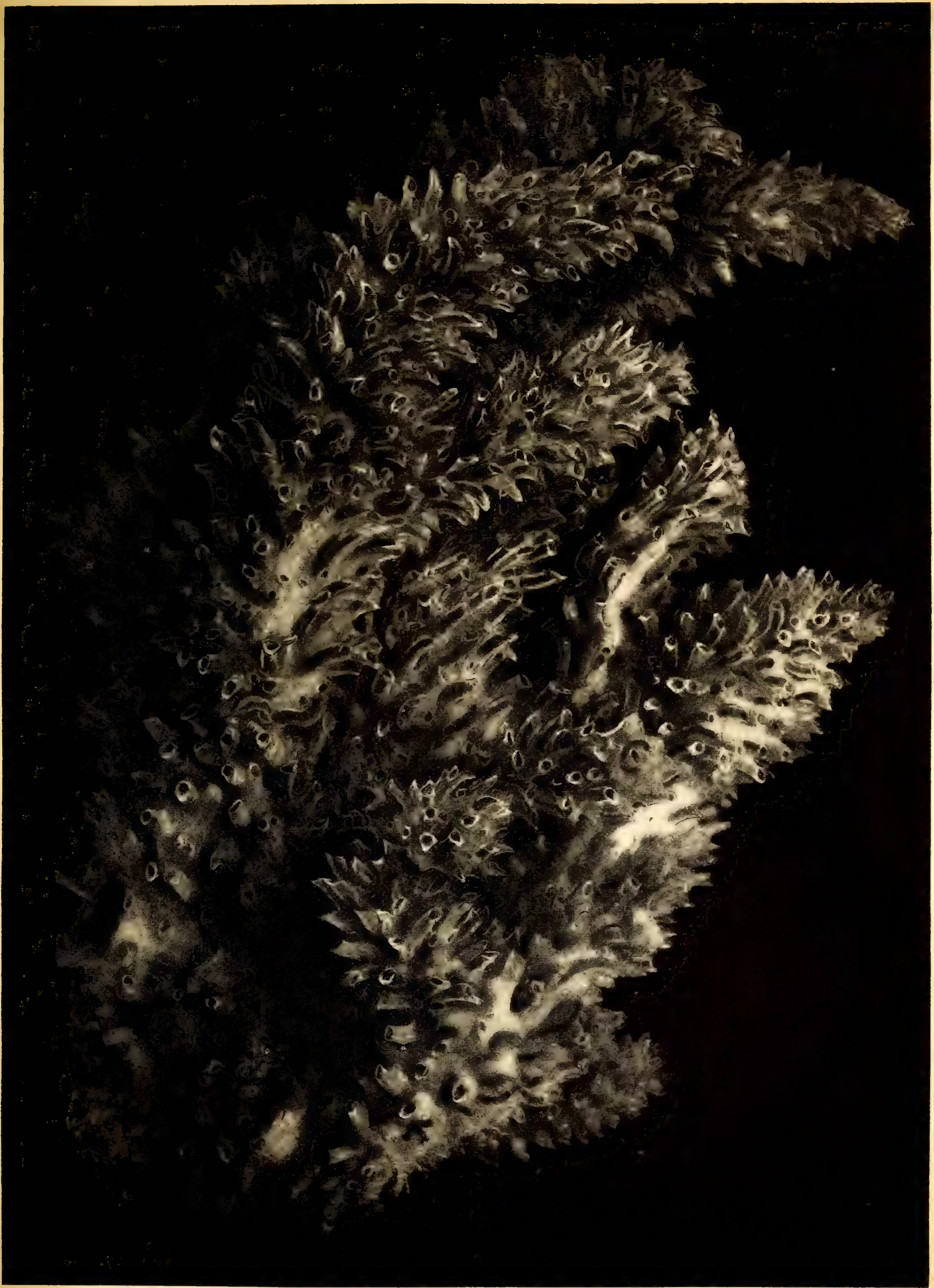
[Plate 8.]

The material here described was dredged by the "Discovery," on January 13, 1902, in 100 fathoms, off Coulman Island, near Victoria Land, in the Antarctic Ocean, and was brought home with the rest of the collections, arriving at the Natural History Museum in September of last year. It had not been possible for the naturalists on the expedition to examine this organism in the living state, and its nature had not been determined until it came into my hands.

The colony is massive, the test nearly transparent, somewhat opalescent, and with a slight yellowish-brown tint. The largest piece in the collection measures roughly 190 by 115 mm. and has twelve branches. This piece is reproduced of natural size in Plate 8. The largest single branch is 90 mm. long and 32 mm. across. The branches are roughly cylindrical in shape, the larger ones are blunt-ended, the smaller ones taper towards their extremities.

Opening at fairly regular intervals over the surface of the colony are the tubes in which polypides dwell, and the substance of the test is sufficiently transparent to enable one to trace the tubes inwards for a moderate distance with the unaided eye, and to recognise the polypides within the tubes.

The margin of the opening of each tube is produced into a blunt lip, and the roughness of the surface of the colony is mainly due to these projecting lips. Each tube contains but one full-grown polypide and its buds, and does not communicate with the other tubes of the colony. The deep or blind end of the tube shows a number of thin septa, hemispherical or irregular, which



Cephalodiscus nigrescens, Lankester.

(Photograph of a portion of a colony of the natural size.)

being secreted in succession, serve to shorten the tube; the increase in the length of the tube is effected by additions of "test" to its free margin and over the whole surface of the colony. The tubes do not branch, and the length of the inhabited part of each is about 10 or 12 mm., and the width 1·2 or 1·3 mm.

The polypides are deeply pigmented and appear black to the naked eye. The pigmented cells are superficial, and are in reality brownish-yellow cells with one or two black spots of small size. The brownish-red patches which in *C. dodecalophus* are found around the oviducts, and the red curved line that passes across the buccal shield, are present also in the new species.

The polypide is about three times as long as that of *C. dodecalophus.* The length of the body from the front of the buccal shield to the end of the visceral mass is 4·5 mm., whereas in *C. dodecalophus* the corresponding measurement is 1·5 mm. The body is about 1 mm. wide, and fits fairly closely in the tube.

Each polypide has from two to nine buds of various sizes attached by longer or shorter stalks to the extremity of its stolon. The stolon is short and stout, and in most of the polypides is directed parallel to the long axis of the body, and away from the plumes.

There are 14 plumes in most of the individuals, but the number varies from 12 to 16. The axes of the plumes are broad and massive and of a black colour, and they do not terminate in the nearly spherical swellings that are found in the Challenger species *C. dodecalophus.* The pinnules are numerous and closely set, and they are not black, although microscopic examination shows that some of the pigmented cells are present on them.

The stomach is not dilated and globular as it is in *C. dodecalophus;* it possesses a pointed cæcum which passes up between the pharynx and the intestine and terminates between the gonads. The gonads consist either of two ovaries, of two testes or of an ovary and a testis. The three kinds of individuals are not distinguishable by any external features, and are not restricted in their distribution; the same branch of the colony may have male, female, and hermaphrodite individuals, and no distinction can be drawn as regards sex between the individuals found in the basal, middle and more terminal portions of the same branch.

The cœlom is divided, as in *C. dodecalophus,* into a pair of large abdominal cavities, a pair of collar cavities opening by collar pores close to the gill slits, and an unpaired cavity in the buccal shield opening by a pair of "proboscis pores" almost immediately above the stalk of the shield.

This new species of *Cephalodiscus* is clearly marked off from *C. dodecalophus* by the massiveness of the colony, the blackness and the large size of the polypides, and the restriction of the polypides and their buds to separate tubes. I propose for it the name *Cephalodiscus nigrescens.*

EXPLANATION OF PLATE.

Photograph of the Natural Size of a Specimen of *Cephalodiscus nigrescens*, Lankester, from the Antarctic Ocean.

Experimental Researches in Vegetable Assimilation and Respiration. IV.—A Quantitative Study of Carbon-Dioxide Assimilation and Leaf-Temperature in Natural Illumination.

By F. Frost Blackman, D.Sc., Fellow of St. John's College, Reader in Botany in the University of Cambridge, and Gabrielle L. C. Matthaei, B.A., Fellow of Newnham College.

(Communicated by Francis Darwin, For. Sec. R.S. Received April 11,—Read April 13, 1905.)

CONTENTS.

Page
Section I.—Introduction. Apparatus and Procedure 402
" II.—On the Internal Temperature of Leaves exposed to Natural Illumination 406
" III.—Assimilation in Natural Illumination 412
" IV.—Illumination and Temperature as "Limiting Factors" in Assimilation 426
" V.—The Photosynthetic Value of Full Insolation 435
" VI.—The Specific Assimilational Characteristics of Diverse Leaves 444
" VII.—The Limitation of Assimilation by the Natural Environment 449
" VIII.—Conclusions 456

Section I.—*Introduction. Apparatus and Procedure.*

It has been made evident by the experiments recently published by one of us,* that the amount of carbon-dioxide assimilation which a leaf is actually performing, or is capable of performing, is profoundly affected by the temperature of the assimilating cells.

Neglect of this factor has been a fruitful source of confusion in attempts to estimate the effect of different intensities of light upon the process of assimilation.

---

* "Experimental Researches on Vegetable Assimilation and Respiration. III.—On the Effect of Temperature on Carbon-dioxide Assimilation," by G. L. C. Matthaei, 'Phil. Trans.,' B, vol. 197, 1904; to be referred to as "Assim. and Resp. III."

Particularly is this the case when *natural* illumination is being investigated, for the diffuse light of the sky produces but little heating effect, while direct solar radiation may heat up a leaf considerably.

During the summer of 1904 we have been working at the relation between carbon-dioxide assimilation and the intensity of natural illumination. For this work we determined the real internal temperatures of our assimilating leaves by the thermo-electrical method described in "Assim. and Resp. III," p. 76. Without the exact data as to the relation between temperature and assimilation set out in that paper, and without the knowledge gained when working with artificial light, we should have been quite baffled in our attempt to deal in detail with all variations of natural light.

In several directions the present paper is of a "preliminary" nature, and we hope to complete the work next summer.

We determined, at the outset, to work in the open air, so as to be able to use direct sunlight, and to avoid the use of heliostats and reflecting silver surfaces.*

The general experimental and analytical procedure has been the same as in "Assim. and Resp. III."

As it was, however, impossible to work sensitive aspirators and a galvanometer satisfactorily when they were exposed to direct or intermittent sunshine, these parts of the apparatus were set up in a north room of the laboratory. Thence they were connected by 50 feet of tubing and wires to the leaf chamber, and to the baryta-absorption-tubes situated on a table on the flat roof of the new University Botany School. On this spot sunshine, when vouchsafed, is continuously available, without any interfering shadows, for quite 12 hours daily, in the height of summer.

In addition, gas for heating, and running water for cooling are there conveniently to hand, so that the temperature of the leaf in its chamber can be fully controlled. Without all these facilities, such as are afforded by very few botanical laboratories, our particular work would have been impossible.

The leaves employed were those of cherry-laurel and of *Helianthus tuberosus.* With the former we have worked for some years, and the latter were employed in order to test whether such a different type of leaf would give similar results.

The cut leaf is set up in the usual flat leaf-chamber with a fixed glass front and an adjustable glass back, through which pass the wires from the thermo-junction in the leaf to the galvanometer.

---

* Langley ('Phil. Mag.,' 1889, p. 10) has shown that the various solar rays are reflected in slightly different proportions by polished silver, and also that the least tarnishing is a serious disturbing factor.

The chamber is submerged in a fixed position on a wooden frame in a large rectangular glass cell full of water (a polished museum-jar measuring 12 in. × 7·5 in. × 3·5 in.); see fig. 1.

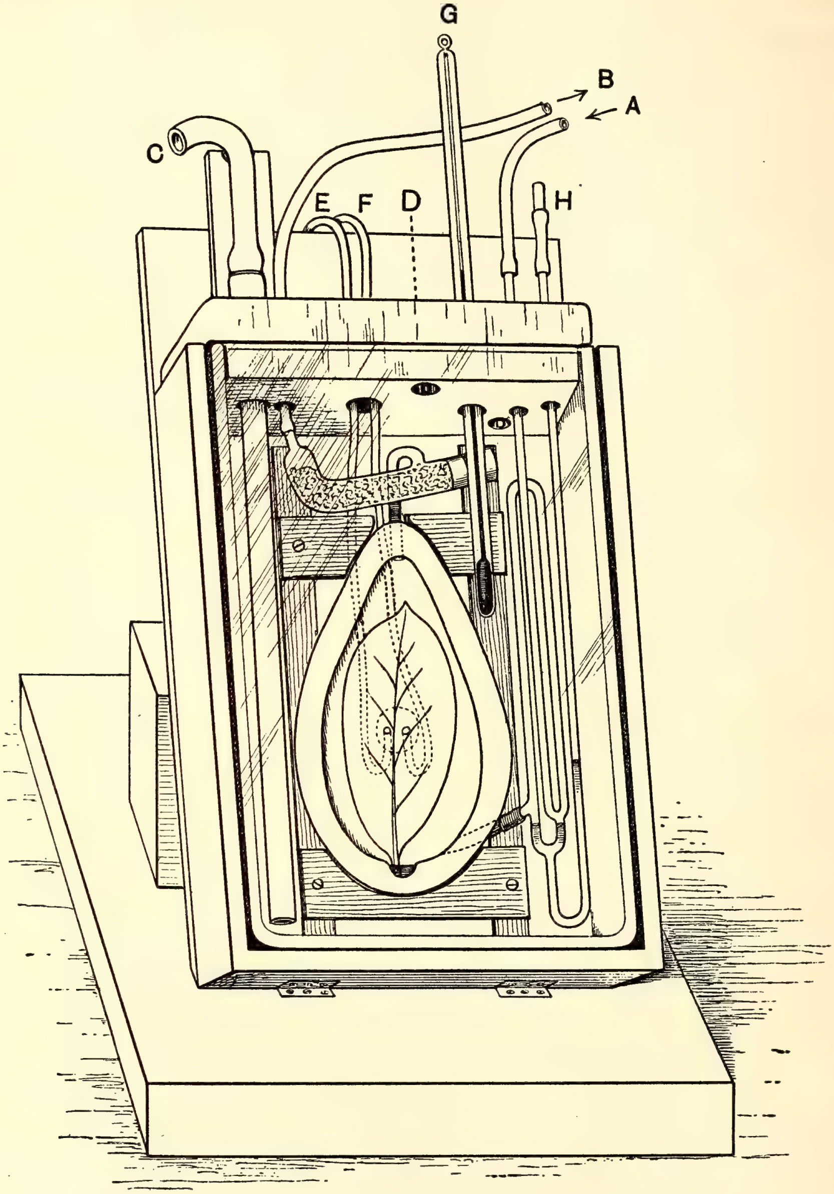


Fig. 1.

This water-bath is closed above by an oblong thick cork cut to fit the mouth. Through appropriate holes in the cork pass:—the air-current tubes, from the CO_2-generator to the chamber (A), and from the chamber to the Pettenkofer tubes (B); the fine rubber tubes (E) and (F) to the back of the chamber, containing the electric wires; an inlet tube (C) from the water supply, passing to the bottom of the bath, and wide enough to carry a rapid circulation of water; holes (D) for the outflow of the water, which was allowed to stream down the back of the bath; and (G) a thermometer, to indicate the bath-temperature. The air-current which enters at (A) passes through a thrice bent glass tube, to give it time to acquire the bath-temperature. The end (H) of this system of glass tubes can be opened when required to let water into it, and this water gravitates at once into the chamber to keep up the supply for the leaf.

The air-current leaving the leaf-chamber by the tube at its apex, passes through a tube of calcium chloride to be dried; condensation in the cooler tube outside the bath is thus prevented.

The water-bath fits into a wooden support resembling an upright box without front or top, painted black inside, and hinged by its lowest edge to a substantial base-board. This hinge permits movement in a vertical plane ranging from the horizontal to the vertical positions. The base-board can be rotated horizontally on a central pivot fixed on the table. By these two movements the bath with the contained leaf-chamber can be adjusted by hand so as to be kept at right-angles to the incident sun's rays throughout an experiment.

The necessity for tilting the bath to follow the vertical displacement of the sun made it difficult to heat from beneath with a burner; the heating was therefore achieved by carrying the water-current, before it reached the bath, through a laboratory water-heater of the horizontal flanged-tube pattern. By varying the amount of gas burning and the rate of the water-flow any temperature above the summer temperature of the running water could be obtained in the bath.

The water-heater was carefully boxed in so as to be protected from the wind, and a subsidiary thermometer was placed in the water-current close to the exit from the heater. This did away with the risk of excessive alterations of temperature, as it gave immediate notification of the full effect of any readjustment of the heating long before the large mass of water in the bath, and the bath-thermometer, reached the corresponding temperature.

As the leaf in its chamber was only two inches from the front of the bath, it received, when exposed freely, the diffuse light from more than a third of the hemisphere of the sky, which at this level, on the roof, is very little interrupted by the tops of adjacent trees.

When it was desired to expose the leaf to the sunlight alone without the general diffuse light, a wooden tube of somewhat bigger section than the leaf, and about four feet long, was fitted tightly on to the front of the bath so as to cut out practically all the diffuse light. An oblique observation-hole cut near the base of the tube allowed the illumination of the leaf to be inspected. For exposure to diffuse light alone, while the sun was shining, a thin board, about 18 inches square, fixed obliquely at the end of a 20-foot batten, was supported high up in the air so as to cast a shadow on the leaf. This hindered the access of only a small portion of the diffuse sky light, but had, of course, to be carefully watched and shifted by hand that a shadow might always be kept on the leaf.

For certain critical experiments it became essential to have known fractions of the sun's luminous radiation falling upon the leaf. An account of the method adopted to ensure this will be found in Section V.

Section II.—*On the Internal Temperature of Leaves Exposed to Natural Illumination.*

In a previous paper* we have shown that the radiation from a high-pressure incandescent gas-light of nominal 1000 candle-power, causes a considerable rise in the internal temperature of leaves exposed close to it even after the radiation has passed through a thick stratum of water.

It becomes, therefore, important to determine what heating effect natural illumination, and, in particular, full normal insolation will produce.

The greater part of the dark-heat radiation from a source of light would be absorbed by interposed water and glass, but a further amount of radiation, not utilisable for the chemical work of assimilation, is absorbed by the more opaque leaf and some excess temperature of the leaf over its environment is inevitable with powerful light.†

With leaves exposed to bright light in a slow current of damp air there is not very much cooling of the leaf by evaporation and the internal leaf temperature is a factor that cannot be ignored.

In the present section we shall consider, in some detail, a few cases which illustrate the temperatures that can be attained inside leaves exposed to various degrees of natural illumination, on the one hand when the leaf is in the open air, and on the other hand when it is enclosed in a glass chamber, with or without water-cooling. The temperatures are in all cases determined thermo-electrically.‡

---

* "Assim. and Resp. III," p. 75.

† A crystalline transparent alum-plate would cut off somewhat more heat, but this is an impracticable expedient, while alum solution is rather less efficient than water, *cf.* Shelford Bidwell, 'Brit. Assoc. Reports,' 1886, p. 309.

‡ For details of procedure, see "Assim. and Resp. III," p. 76.

We will take first two special experiments with leaves of cherry-laurel in the open air, and subsequently one in which the leaf is in a glass chamber, sunk in a water-bath (the normal arrangement in our assimilation experiments).

For the experiments in the open air, the cut leaf was pinned out by its margin on a small thin rectangular board which was hinged by its lower edge to a large rigid horizontal base-board. Through the thin hinged board was an oval opening, only a little smaller than the leaf, and over this the leaf was stretched.

The stalk of the leaf dipped into a little well of water in the base-board and remained in the water at whatever angle the hinged frame might be sloped. A thermo-junction was inserted in the mid-rib of the leaf and the free ends of the wires hung down into mercury-cups on either side of the water-well. Supported in a hole through the base-board was a funnel which contained a thermometer and the control thermo-junction, and could be filled with water at any desired temperature. The whole of this part was carefully screened from solar or indirect radiation. The two junctions were connected up with one another and with the galvanometer by wires running to mercury-cups in the horizontal board.

On July 17, 1904, the sun shone with unclouded brilliance throughout the day. A leaf was set up on the hinged frame and the following temperature observations were made by means of the galvanometer.

Hour.	Conditions.	Leaf-temperature.
A.M.		° C.
11.25	Leaf in shade; shade-temp., 27° C.	28·0
11.50	Leaf vertical, facing south in direct sun. Bright mercury thermometer in sun, 30°·5 C.	38·3
11.53		39·8
11.55		39·3
P.M.		
12.7	Frame inclined till leaf is normal to the solar radiation	44·6
12.10	A glass plate interposed in front of leaf...............	44·3
12.20	Glass plates also behind, above, and at sides, within an inch of leaf, boxing it in	51·0
12.25	Visible brown spot of killed cells.	
12.28	Sun-temp., 32° C. Front and top glasses off, others remaining	44·5
12.35	All glass plates taken away................................	41·4
12.38	Leaf-frame placed vertically	39·0
12.45	Large rectangular cell of water (temp., 23° C.) close to leaf in front, leaf closed in behind, above, and at sides with glass plates	41·8
	Experiment ended as leaf is extensively turning brown, killed by the heating to 51° C.	

Hour.	Conditions.	Leaf-temperature.
P.M.		° C.
2.30	A new leaf set up. Leaf in shade.	
2.40	Temp. in shade of chimney, 27°·3 C. (Sun-temp., 32° C.)	28·1
2.45		28·1
2.50		28·6
3.0		28·6
3.10	Sun's radiation reflected on to the leaf from plane glass mirror	33·0
3.17	Ditto, but water-cell in path of reflected rays	30·4
3.20		29·6
3.22	Mirror removed	28·4
3.23	Water-cell removed	28·2
3.24	Mirror replaced without water-cell	30·1
3.27	Substituted a mirror of thin glass	31·0
3.30	Mirror removed	28·0
3.34	Leaf moved into direct sunshine, leaf vertical	31·7
3.36		33·6
3.39	Leaf at right angles to solar radiation. Sun-temp., 29°·4 C.; shade-temp., 26°·7 C.	33·6
3.47	A cell full of water (25° C.), 1 inch in front of leaf	31·3
3.50	All shaded with black cloth	26·6
4.0	Leaf moved into shade, boxed in with glass plates. Shade-temp., 26°·3 C.	28·4
4.4		28·5
4.6	Sun reflected by mirror on to boxed-in leaf in shade	36·5
4.10	Back glass alone removed. Shade-temp., 25°·3 C.	30·7
4.20		29·9
4.27	Leaf vertical in direct sun with front glass on alone	33·2
4.33	Leaf boxed in by adding the other glass plates	42·6
	Experiment stopped. Shade-temp., 25°·2 C.	

We may sum up these observations as follows:—

Experiments in Direct Sunshine.

Excess of Internal Temperature of Leaf in Brilliant Sunshine over Reading of adjacent Bright Mercury Thermometer in the Sun.

Hour.	Conditions.	Excess leaf-temperature.
		° C.
Noon	Leaf vertical	8—7
,,	Leaf normal to sun's rays	13—9
,,	Leaf normal in glass chamber	20
,,	Ditto, behind water-cell	10
About 4 P.M.	Leaf vertical	2—5
,,	Leaf normal to sun	4
,,	Ditto, behind water-cell	2
,,	Leaf normal to sun, in glass chamber	14

Experiments in Shade.

Excess of Internal Leaf-Temperature in Shade, on Cloudless Day, over adjacent Thermometer Reading.

Hour.	Conditions.	Excess leaf-temperature.
		° C.
Noon	Leaf in open air	1
3.30 P.M.	" "	1—1½
"	Reflected sun on leaf	6
"	Reflected sun through water	3
"	Leaf in glass chamber	2
"	Ditto, reflected sun on chamber	10

The raising of the leaf-temperature in a leaf surrounded with glass plates when lighted by direct or reflected sunshine is very striking, and we see why observers have always failed with attempted estimations of assimilation in bright direct sunshine when the procedure has been simply to place the leaf in an unprotected glass tube in the sun.

With adequate water-cooling the tube could be exposed to the sun without killing the leaf, but even then the temperature of the leaf would be very much raised.

This heating up of the leaf will, no doubt, partly be due to checking of transpiration in the enclosed space, but the effect comes on so quickly that it must chiefly be due to "the greenhouse effect," the imprisonment of the reflected dark-heat rays by the glass plates which are almost impervious to them.

We may now give some details about the internal temperature of a cherry-laurel leaf placed, as in our assimilation experiments, in the flat glass "leaf-chamber," and this sunk in a large glass bath of running water, *cf.* fig. 1.

The chamber was adjusted at right angles to the sun's radiation and the leaf-temperature was determined at the galvanometer at intervals of exactly one minute. At the same moment the state of the natural illumination was written down by an independent observer. The observations were made on July 20, 1904. Clouds were drifting irregularly across the sun and the determinations start at 12.4 P.M., just as a large cloud cleared away from the sun. It may be stated at once that there is a lag of about one minute between the change of insolation and the temperature-change that results, so that in the table the temperature at the subsequent minute really corresponds to each particular illumination:—

Temperature of water-bath.	Time.	Illumination.	Leaf-temperature. °C.
Bath-temp., 18°·6 C., water circulating quickly	12.4	Brilliant sun	22·4
	.5	Moderate sun	27·3
	.6	Brilliant sun	27·8
	.7	Moderate sun	29·0
	.8	Bright sun	26·7
	.9	Brilliant sun	24·6
	.10	Very brilliant sun	28·3
	.11	Thin cloud	29·0
	.12	Thick cloud	24·9
	.13	Thicker cloud	23·4
	.14	Sun quite obscured	22·3
	.15	Thick cloud	21·8
	.16	Thin cloud	22·7
	.17	Sun quite obscured	21·9
	.18	Thick cloud	22·2
Bath-temp., 18°·4 C.	.19	Sun quite obscured	21·9
	.20	Sun gleam	24·3
	.21	Thick cloud	22·5
	.22	Thin cloud	22·5
	.33	„	23·9
	.34	Moderate sun	24·3
	·35	Bright sun	25·4
Bath-temp., 18°·8 C.	.35½	Surface darkened with cloth	
	.36	Dark superficially	24·7
	.37	„ „	20·2
	.38	„ „	19·4
	.39	„ „	19·4
	.40	„ „	19·3
Bath-temp., 18°·8 C.	.40½	Cloth removed.	
	.41	Brilliant sun	22·3
	.42	Bright sun	27·7
	.43	Brilliant sun	27·9
	.44	„	30·0
	.45	„	30·7
Bath-temp., 19°·3 C.	.46	„	29·7

These readings show how decidedly the temperature of a leaf under experimental conditions is affected by variations in the intensity of solar radiation: while the bath (through which, of course, water is running rapidly) only varied in temperature 1° within the 40 minutes, the leaf-temperature oscillated up and down with the varying fine shades of natural illumination through a range of 9°.

If the natural light is variable, very frequent galvanometric readings must be taken to arrive at a real average temperature for a given assimilation-reading. When a knowledge of this is important, and the light is variable, we have taken the temperature as often as every 3 minutes, all through the experiment.

The difference between the observed temperature of the water-bath and the determined temperature of the leaf gives a rough practical measure of the intensity of the radiation falling on the leaf, and in some experiments this has been calculated for each reading, and used to elucidate the contemporaneous assimilation-values.

If the temperature of the bath and also that of the leaf be plotted graphically, as in figs. 4 and 5, we get in their difference a continuous record of the approximate light intensity.

During all the later experiments, records of the air shade-temperatures and of the readings of a black-bulb-in-vacuum thermometer in the sun have been kept, but we have not thought it important to publish these.

A single extract (Experiment VI, July 24, 1904) may be given here to show the relation of the whole set of temperatures.

Time.	Illumination.	Shade-temperature.	Bright mercury thermometer in sun.	Vacuum thermometer in sun.	Bath-temperature.	Leaf-temperature.
A.M.		° C.	° C.	° C.	° C.	° C.
11.23	Dull	23·9	24·5	36·5	19·7	24·3
11.30	Gleams of sun	24·1	24·8	37·5	19·7	22·4
11.44	Fair sun	24·8	25·5	42·0	20·2	26·9
11.53	Brilliant sun	25·8	28·0	54·5	20·6	27·0
P.M.						
12.2	Dull	24·7	24·6	39·0	19·7	21·5
12.20	Steady dull	24·0	25·1	37·5	19·8	21·5
1.10	"	23·8	24·0	29·5	19·3	21·5
1.20	Clearing	24·5	24·7	36·0	19·7	21·9
1.31	Sun coming out	25·4	26·4	41·0	20·0	25·4
1.42	Bright sun	26·1	27·4	48·5	20·6	28·4
1.51	Sun and thin cloud	25·7	26·7	50·0	20·8	27·4

However obscured the sun may be, it is always found that the diffuse light of the sky, according to its brightness, heats up the leaf 1°, 2°, or even 3° above the temperature of the bath.* This demonstrates the greater effectiveness, in absorption of radiation, of the coloured and semi-opaque leaf over the colourless and transparent glass and water.

Such high temperatures in ordinary leaves in the sun are somewhat unexpected.* For succulents, and for them only, have such temperatures been previously demonstrated.†

---

* It is only after darkening the water-bath with a black cloth that the temperature of the leaf falls as low as that of the bath.

Besides a profound influence on respiration, and hence on assimilation, this internal heating up of leaves in bright light may have an important and hitherto unrecognised bearing on quite other phenomena, such as the success or failure of inoculation of leaves by the germ-tubes of parasitic fungi, etc.

Section III.—*Assimilation in Natural Illumination.*

We now pass to a consideration of the relation of leaf-temperature and intensity of illumination to the observed values of assimilation under various natural conditions.

A brief account of the relation of CO_2-assimilation to temperature must be given first.

In "Assim. and Resp. III" there have been given full data, and also the first principles of this relation. A further theoretical consideration of these principles will be found in a contemporary article by one of us.‡ We may here sum up as much as will concern us.

1. For each temperature at which the assimilating cells of a leaf may find themselves, there is a particular maximal amount of assimilation possible. No increase in the amount of incident light or of available CO_2 will cause the leaf to assimilate more if the temperature remains unchanged. If there is not enough CO_2, or not sufficient light available, then one of these becomes a "limiting factor," and the maximal assimilation for the temperature cannot be attained, but some smaller amount only.

2. The temperature-maxima for assimilation increase rapidly as one ascends the temperature scale, the relation between temperature and assimilation being very similar to the relation between temperature and respiration.

3. At moderate temperatures a leaf can maintain its assimilation at the maximal value continuously for a considerable time, but at higher temperatures (towards 30° C. for cherry-laurel) the initial maximal value is not maintained; instead, a regular falling-off sets in.

4. This decline from the initial value is, for each temperature, more rapid at first than subsequently. The higher the temperature the more precipitous is the whole declining curve. These phenomena necessitate the introduction of a "time-factor" into assimilation values at high temperatures.

The added complication of a time-factor makes critical work with varying light and other varying factors very difficult at really high temperatures. These relations will form the subject of a separate communication.

---

* See note A on p. 459.

† Askenasy, 'Botan. Zeitung,' 1877.

‡ F. F. Blackman, "Optima and Limiting Factors," 'Annals of Botany,' vol. 19, April, 1905.

In the present work the temperature of the leaf rarely exceeds 30° C., and the duration of the individual experiments is usually not long, so that not much correction need be made for the time-factor. It will be mentioned when this becomes necessary.

It will be convenient to state for future reference what amounts of assimilation we shall take as maximal for the different temperatures concerned in this work.

The data for cherry-laurel are to be found in "Assim. and Resp. III." The final curve, given in fig. 6 of that paper, was smoothed out by free-hand to the curve C in fig. 2 of this paper. The data used for it represent the assimilation-values two hours after the initial moment of heating to the required temperature. The *initial* values will, at high temperatures, be greater, and their precise value depends partly on a hypothesis discussed elsewhere.* These initial values are represented by the curve B, which below 25° C. coincides with the other curve, C.

The values for each degree of the curve C are set out below, being expressed in grammes CO_2, assimilated per 50 sq. cm. per hour.

° C.		° C.		° C.		° C.	
9......	0·0038	16......	0·0065	23......	0·0103	30......	0·0151
10......	0·0041	17......	0·0070	24......	0·0109	31......	0·0160
11......	0·0045	18......	0·0075	25......	0·0115	32......	0·0171
12......	0·0049	19......	0·0080	26......	0·0121	33......	0·0182
13......	0·0053	20......	0·0085	27......	0·0128	34......	0·0193
14......	0·0057	21......	0·0091	28......	0·0135	35......	0·0205
15......	0·0061	22......	0·0097	29......	0·0143		

The curve A represents the curve of initial assimilation-maxima for *Helianthus.* It is based on such observations in the present paper as are undoubtedly maximal. Four of these are above all suspicion, as follows:—

	° C.
Experiment X	0·0090 at 18·0
" XI...............	0·0109 at 20·7
" XI...............	0·0131 at 22·3
" XVI	0·0290 at 30·0

The values for the two leaves are fairly close together at 18° C., but at 30°·0 C. *Helianthus* has gone up out of proportion to cherry-laurel, *i.e.*, it has a larger coefficient of temperature-acceleration.

---

* F. F. Blackman, "Optima and Limiting Factors," *loc. cit.*

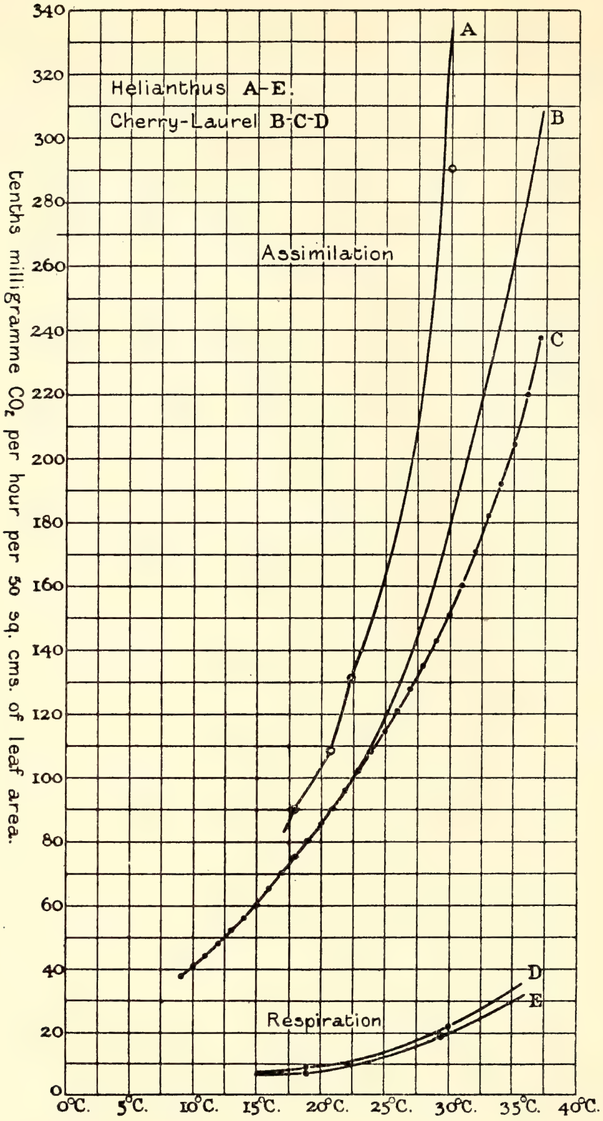


Fig. 2.

The curves D and E represent the respiration of the two leaves, cherry-laurel and *Helianthus* respectively, at the various temperatures, expressed in grammes CO_2 per 50 sq. cm. leaf-area per hour. The values determined were as follows:—

Helianthus......... Experiment IX, 0·0018 at 29°·5 C.; Experiment XVI, 0·0007 at 19°·0 C.

Cherry-laurel...... Experiment XIII, 0·0021 at 30° C.; Experiment II, 0·0009 at 19°·0 C.; and Experiment III, 0·0008 at 18°·4 C.

In the experiments in "Assim. and Resp. III," it was demonstrated for cherry-laurel that the assimilation-maxima at a given temperature were practically identical for all similarly treated leaves of the same age on the same evergreen bush at any one season of the year. In July and August, 1904, when our new experiments were performed, the cherry-laurel bears new leaves just come to maturity and also leaves of the previous year. Experiment showed that the latter have by now quite low assimilation temperature-maxima, but that the newly matured leaves give the same values as we found in April, 1902, and April, 1903. This agreement for three separate years gives one considerable confidence in this material.

Occasionally a leaf will show marked individuality and give aberrant values, but this is very rare; Experiment XII furnishes a case.

Let us consider firstly some preliminary experiments with various natural variations of illumination.

As the object of this work has been to determine completely the relations between assimilation and all intensities of natural illumination, and, as our previous work has shown that it would be fallacious simply to regard a succession of assimilation readings in a succession of different illuminations as being solely determined by the light, it therefore becomes essential to make inquiry about each assimilation-value obtained, and to determine (either experimentally or statistically) whether the assimilation is or is not being limited by the temperature of the leaf to a smaller amount than the light would provide. In the early experiments this point is not examined experimentally, but is argued out by reference to the data on p. 413, and sometimes to data obtained in subsequent experiments.

In the first four of these experiments on illumination, the actual leaf-temperature was not determined thermo-electrically, but has been subsequently roughly estimated by adding to the temperature of the bath such allowance as later exact experiments showed to be appropriate for each condition of illumination.

Experiment I.—Cherry-laurel; leaf of current year set up in the leaf-chamber during the night in bath with moderate flow of water through it, and no artificial heating throughout the experiment. The bath at first vertical and superficially darkened with black cloth. From 4.15 to 5.15 A.M., an estimation of the darkened leaf was taken, which shows an apparent minute absorption of CO_2. This is probably somewhat in error, as a slight respiratory production of CO_2 would have been expected; the chamber was, however, not perfectly dark. As soon as the rising sun shone on the bath—at 6 o'clock—the black cloth was removed, and the bath adjusted by means

Experiment I (July 12, 1904).—Cherry-laurel; Weight, 1·60 grammes; Area, 50·3 sq. cm.; Current Rate, 810 c.c.; $CO_2 = 2·3$ per cent. (average).

Exposure.	Time.	Illumination.	Temperature of bath.	Estimated temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
Leaf-chamber darkened with cloth	A.M. 3.15—4.15	Bright dawn, sunrise 3.58	° 15·5		Preliminary		
	4.15—5.15		15·8 16·1	16·0	0·0346	0·0001	[0·0008]
6 A.M., leaf-chamber lighted; thenceforward adjusted continuously normal to sun, *plus* diffuse light	5.15—6.15 6.0	Sun on chamber...	16·6	16·6	0·0366	0 0014	transition
	6.15—7.15 7·0	,, no clouds ... ,, ,, ...	18·2 20·1	21·1	0·0370	0·0082	0·0092
	7.15—8.15 7.45	,, ,, ... ,, light clouds...	21·7 20·5	25·4	0·0390	0·0104	0·0117
	8.15—9.15 8.50	,, ,, ... ,, cloudless	23·5 24·5	26·7	0·0400	0·0110	0·0126

All amounts of CO_2 are expressed in grammes. The values in the Real Assimilation column are in this and all subsequent cases arrived at as follows:—The value for CO_2 absorbed by the leaf (in previous column) is brought to the value per hour and then reduced to an area of 50 sq. cm. To this is added the respiration value per hour (from the curves D or E in fig. 2) corresponding to the measured or estimated temperature of the leaf. Fifty sq. cm. was chosen as the standard area because it approximates very nearly to the actual sizes of the leaves. When a single temperature only is given for an estimation it may be taken that it is the mean of a number of observations; in a few cases it has been thought worth while to give the details.

of its horizontal and vertical movements until the leaf was normal to the sun's rays. After a quarter of an hour "preliminary" an estimation of the combined effect of low direct sun and diffuse light was made—6.15 to 7.15 A.M. During this and subsequent readings the chamber was readjusted to face the sun every 10 minutes.

Throughout the series of readings the temperature of the bath rose continually, as the sun, shining on the supply-water-pipe and on the bath, gained in power; the rate of water-flow remained unaltered.

The graphic representation, fig. 3, allows us to gather an idea of the rapid mounting up of early morning assimilation. It is not true, however, that the actual amounts are measures of the increase of light. Subsequent work made it probable that the temperature would be the limiting factor in this case, the light being in excess. In Column 5 are given the temperatures for which the observed assimilations are maximal; we find that they agree well with our other experiences of how much such insolation heats up a leaf. The assimilation values are in this case to be taken as a direct measure of the leaf-temperature.

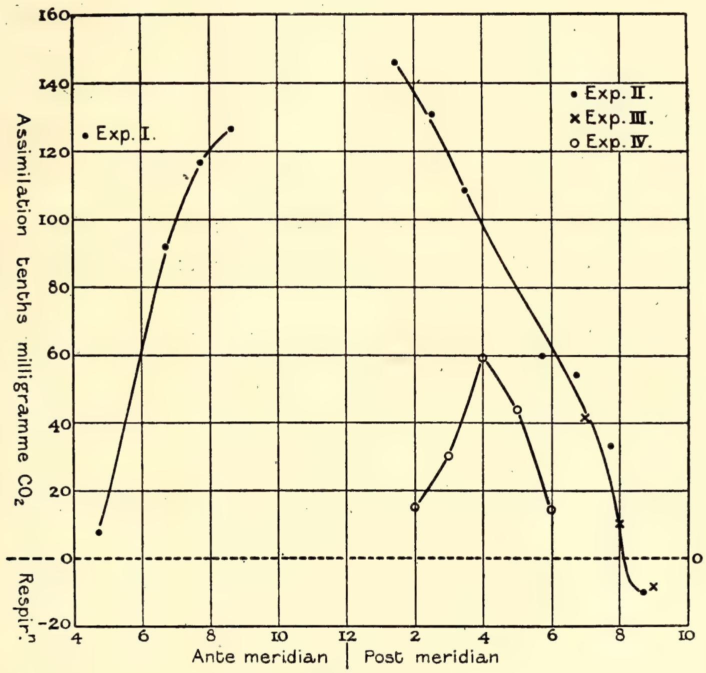


FIG. 3.

In these early experiments we rather interpret than prove the significance of the assimilation numbers. Direct proof that such and such effects are due to light or to temperature as limiting factors will be found in later experiments.

Experiment II deals with exposure of a leaf "in the shade" to the collective diffuse light of the sky, the direct sunlight being always cut off by the shadow-board. In this experiment, and in this one alone, the leaf-chamber was not in the bath, but was placed horizontally in the free air with thermometers in contact with it to give a rough idea of the shade-temperature around the leaf. The temperature of a leaf within a chamber in the shade is quite 2° above that of the mercury thermometer (see p. 409). We may thus assume in the first reading the leaf to have been at about 29° C., and we find that the assimilation is maximal for 29°·4 C. (*cf.* p. 413); evidently the diffuse light in the shade is more than enough for an assimilation of 0·0146, but the temperature limits it to that amount. In the second reading the assimilation is a little lower, while in the third reading it is much smaller, and it is clear that this is due to diminution of light, as the

Experiment II (July 13, 1904).—Cherry-laurel; Weight, 1·70 grammes; Area, 54·3 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 2·4$ per cent. (average).

Leaf exposed to Diffuse Light under Shadow-board all Day. Chamber in Air, not in Bath.

Time.	Illumination.	Temperature of air.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
11.45 A.M.—1 P.M.	White cumuli, with gleams of sun	24°·9 27·0	Preliminary		
P.M. 1—2	,, ,,	27·0	0·0376	0·0137	0·0146
2—3	,, ,, sun 2.20—2.50	27·0	0·0387	0·0123	0·0131
3—4	White cumuli, with gleams of sun	26·6	0·0397	0·0100	0·0110
4—5.15	Mostly sun	26·0			
5.15—6.15	Sun	25·2	0·0376	0·0048	0·0060
6.15—7.15	Sun; cloudless; 6.40, sun below roof	24·3 21·9	0·0390	0·0046	0·0054
7.15—8.15	Cloudless; sunset 8.11 ...	21·9 19·8	0·0386	0·0027	0·0033
8.15—9.15	Dusk, faint clouds	19·8 17·8	0·0367	minus 0·0010	

amount 0·0110 would only be maximal at 24° C. Light is thus falling off faster than the temperature, and has become in its turn the limiting factor; the decreasing assimilation is henceforward a direct measure of the light intensity only.

This series of readings in the waning intensity of afternoon diffuse light is represented graphically in fig. 3, and it will be seen that assimilation is considerable right up to sunset.

The assimilation, of 0·0033, in the last reading but one could have been carried out with a temperature of 7° C. Finally, there is no appreciable light or assimilation, and from 8·15 to 9·15 we get, instead, respiration with an output by the leaf of 0·0010 gramme CO_2.

Experiment III.—The leaf in this experiment was exposed to a natural sequence of irregular illumination throughout the day. The leaf-chamber was inside the water-bath and kept faced normal to the sun (or, when cloudy, to the approximate position where the sun would be) throughout the day. The only interference with the natural sequence of things was an alteration in the temperature of the bath about 4 P.M. The temperature had been kept at about 18° C. by circulating water until 3.35 when heating up (by a primitive and very slow method) was begun. The heating up lasted for two hours, and then the bath slowly cooled down again.

The first three readings—0·0090, 0·0081, 0·0093—are maxima for the temperatures of 21°·0, 19°·1, and 21°·3 C. respectively, and these probably were the average temperatures of the leaf in the respective readings, heated up by the diffuse light and feeble variable sun. Possibly the second reading is really just limited by the dull light. The fourth reading of 0·0067 is clearly so limited as it is well below the maximum even for the bath-temperature. In the fifth reading dull light prevailed at first, but later gave place to sunshine, and the assimilation is 0·0077, a slight increase. During this reading heating up of the bath was begun, but this is not the cause of the rise of assimilation, as 18° C. would have been adequate for what takes place. The sixth and seventh readings both show a large increase due to the improved light, and are a direct measure of this.

In the eighth reading the sun sinks behind a ridge of the roof, and assimilation drops to 0·0043, while there is still appreciable assimilation up to sunset, after which the CO_2 of respiration begins to escape from the leaf.

The values obtained after the sun has gone below the roof are, of course, diffuse light values, and they agree closely with those of Experiment II under the same conditions, as fig. 3 shows.

Experiment III (July 14, 1904).—Cherry-laurel; Weight, 1·75 grammes; Area, 55·1 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 2{\cdot}5$ per cent. (average).

Leaf-chamber adjusted Normal to Sun throughout the Day.

Time.	Illumination.	Temperature of bath.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
A.M. 9.35—10.35	Bright sunshine …………	18°·1	Preliminary		
10.35—11.35	Light clouds ………………	18·2 17·4	0·0392	0·0089	0·0090
11.35 A.M.—12.35 P.M.	Grey clouds; then less dull	17·4 18·7	0·0387	0·0079	0·0081
P.M. 12.35—1.35	Sun; cloudy ………………	18·5 18·0	0·0415	0·0092	0·0093
1.35—2.35	Mostly sun ………………	18·2 18·8			
2.35—3.35	Dark clouds ………………	18·6	0·0396	0·0064	0·0067
3.35—4.35	Overcast till 4.5; sun ……	18·5 23·5	0·0398	0·0071	0·0077
4.35—5.35	Sun behind chimney; last half hour, sun	23·5 26·6	0·0405	0·0103	0·0111
5.35—6.35	Sun through thin cloud …	26·6 24·4	0·0405	0·0100	0·0108
6.35—7.35	Sun below roof; thin cloud	24·4 22·7	0·0390	0·0033	0·0043
7.35—8.35	Sunset 8.11 ………………	20·0	0·0401	0·0002	0·0010
8.35—9.35	Dusk ………………………	18·4	0·0376	minus 0·0009	

Experiment IV is the first with a leaf of *Helianthus tuberosus*. This leaf had been set up for an experiment on the previous day with a thermo-junction in it, but owing to trouble with the galvanometer-leads the experiment was abandoned, and re-started the next day with the same leaf still in the chamber.

On this afternoon took place the most violent rain and thunder storm of the year, and assimilation estimations were taken through it. The first reading shows how small is the assimilation, 0·0015, during the gathering of the dense leaden clouds that preceded the storm. A higher number is reached during the bursting of the storm and the slow clearing up.

Experiment IV (July 30, 1904).—*Helianthus tuberosus;* Weight, 1·47 grammes; Area, 70·1 sq. cm.; Current Rate, 800 c.c.; CO_2 = 2·5 per cent. (average).

Leaf-chamber faced towards the Approximate Position of the Sun, at Intervals.

Time.	Illumination.	Temperature of bath.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
P.M. 12.30—1.30	—	° —	Prelim	inary	
1.30—2.30	Heavy leaden clouds; storm drifting up	18·2	0·0426	0·0011	0·0015
2.30—3.30	Violent thunderstorm at first, then slowly clearing up	18·3	0·0384	0·0032	0·0030
3.30—4.30	Brighter; no rain............	18·3	0·0386	0·0073	0·0059
4.30—5.30	Sun at first, then clouded over; storm drifting up	18·3	0·0394	0·0050	0·0043
5.30—6.30	Overcast, steady rain; 6.10, heavy storm	18·0	0·0380	0·0007	0·0010

In the third reading rain ceased, and 0·0059 marks the brighter period. Soon, after a few gleams of sun, a second storm began to drift up, and this burst at the end of the fifth reading. These assimilation values are plotted in fig. 3, and show the passage from one big storm to another through a brighter interval. The assimilation is in no case near the maximum for the temperature, and is a measure of the light only. Possibly the vitality of the leaf has been lowered by prolonged sojourn in the chamber not far from the mercury connecting cups.

We now come to a group of three experiments under the natural sequence of illumination, in which the temperature of the leaf was actually determined thermo-electrically throughout the experiment.

Experiment V took place on a day which was dull up to about 3 o'clock, when the sun came out brightly; at the same time the temperature of the bath was raised by the circulation of hotter water. The leaf is thus in the last reading both hotter and better lighted than in the first three readings, and there is a marked change in the assimilation.

The first three readings give assimilation values of 0·0073, 0·0065, 0·0077, the average temperature of the leaf being about 20°·5 C. Now, the assimilation maximum for 20°·5 C. is 0·0088 (see p. 413), from which it follows that the amount of assimilation is not limited by the temperature, but by the dulness

Experiment V (July 22, 1904).—Cherry-laurel; Weight, 1·80 grammes; Area, 55 sq. cm.; Current Rate, 800 c.c.; CO_2 = 2·4 per cent. (average).

Leaf-chamber adjusted Normal to Sun throughout the Day.

Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
P.M. 11.40—12.40	—	° 19·0	° —	Preliminary		
12.40—1.40	Dull, cloudy sky ...	19·0	20·5	0·0392	0·0070	0·0073
1.40—2.40	" " ...	18·8	20·0	0·0384	0·0062	0·0065
2.40—3.10	Clouds less heavy...	19·2	21·1	0·0192	0·0037	0·0077
3.10—3.40	Sun coming out ...	Heated to 29·0				
3.40—4.25	Bright sun for most of estimation	29·0 26·3	Variable, 35·8—31·5	0·0368	0·0132	0·0190

of the illumination. In the final reading the value obtained, 0·0190, is the standard maximum for 33°·7 C.,* which is about the average observed temperature of the leaf. Here, then, the assimilation, in spite of its magnitude, is yet limited by the temperature and not by the light, which is quite superfluously bright (as will be precisely demonstrated in Section V).

It is interesting to note that the excess of the leaf-temperature over the bath-temperature gives a rough measure of the intensity of the incident light-radiation. The successive values 1°·5 C., 1°·2 C., 1°·9 C., and 5°·2 to 6°·8 C., vary parallelly with the assimilation.

Experiments VI and VII exhibit mixed effects of varying illumination and temperature, and frequent records of both were taken in order to see how far the assimilation values could be explained in detail by these data. We will take first Experiment VI, in which each reading lasted an hour, and the temperature and illumination were noted every 10 minutes. The details of these are given in the schedule of the experiment, and graphically in fig. 4 The significant averages are given in Table I on p. 424.

The day was dull and cloudy; continuous sun about 2 to 3 P.M. only. The assimilations fall short of maximal throughout the day. As the chamber was faced to the south, and left unmoved all day, the afternoon bright sun fell on it obliquely, and did not produce its full effect.

---

* Or for 33° C., as this is an early reading; see p. 412 on influence of "time factor."

Experiment VI (July 24, 1904).—Cherry-laurel; Weight, 1·90 grammes; Area, 62·6 sq. cm.; Current Rate, 800 c.c.; CO_2 = 2·4 per cent. (average).

Leaf faced to South; Position unchanged throughout Experiment.

Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
A.M. 8.30—9.30	Dull, cloudy day ...	° —	° —	Preliminary		
9.30—10.30	Alternate sun-gleams and dull	18·8 19·4	23·5	0·0403	0·0100	0·0092
10.30—11.30	Dull till 11, then faint sun	19·2 20·2	23·2	0·0413	0·0103	0·0095
11.30 A.M.— 12.30 P.M.	Gleams of sun till 12, then dull	20·6 19·5	23·8	0·0431	0·0111	0·0102
P.M. 12.30—1.30	Uniformly dull......	19·7	21·4	0·0410	0·0076	0·0071
1.30—2.30	Bright sun throughout	20·4	26·9	0·0360	0·0114	0·0108
2.30—3.30	Bright sun, except at 2.50, when clouds	20·7	26·3	0·0367	0·0098	0·0093
3.30—4.30	Bright at beginning and at end; dull in middle	19·9	21·8	0·0366	0·0062	0·0061
4.30—5.30	Sun at first, then thin cloud	20·0	21·5	0·0372	0·0054	0·0054
5.30—6.30	Bright sun; sun and thin cloud	19·8 19·7	20·4 19·9	0·0365	0·0040	0·0041

The three first readings have about the same illumination-intensity; witness the uniform leaf-temperature and uniform excess of leaf-temperature over bath-temperature. As the scattered gleams of sun fall on the leaf with decreasing angle of incidence, so the assimilation rises from 0·0092 to 0·0102, and gets practically to be maximal for the third reading. In the fourth reading a long period of very dull sky brings the assimilation down to 0·0071. After that, continuous sun gives the highest assimilation of the day, and the highest excess of leaf-temperature, though a still larger assimilation would be expected. From that point the light steadily becomes feebler, and that part

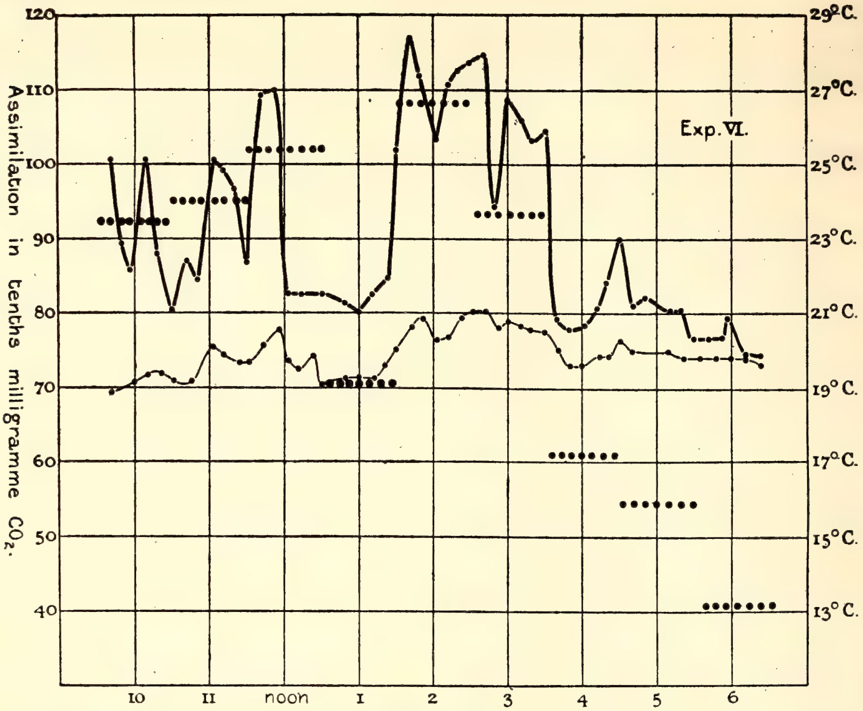


Fig. 4.

The heavy line connects the successive leaf-temperatures; the light line the corresponding bath-temperatures. The horizontal lines of dots indicate the assimilation in successive hours.

Both the temperature-curves show fluctuations according as the sun is clear or clouded, but these are much more violent in the leaf than in the large mass of water in the bath, which is continually being replaced by running water of fairly uniform temperature.

From inspection of the graphic record of the bath-temperature, one can learn when the sun was shining and when clouded; from the graphic of the leaf-temperature a more detailed reconstruction of the sequence of illumination is possible, while the *difference* between the two temperatures gives information of considerable precision about the intensity of illumination; this is used in Tables I and II.

Table I.—Experiment VI.

No. of estimation.	Hour.	Excess temperature of leaf.	Observed leaf-temperature.	Maximal assimilation for observed temperature.	Observed assimilation.	Deficiency of observed assimilation.
1	10	4°·3	23°·5	0·0106	0·0092	−14
2	11	3·6	23·2	0·0104	0·0095	− 9
3	noon	4·1	23·8	0·0108	0·0102	− 6
4	1	1·9	21·4	0·0093	0·0071	−22
5	2	6·4	26·9	0·0127	0·0108	−19
6	3	5·6	26·3	0·0123	0·0093	−30
7	4	2·0	21·8	0·0095	0·0061	−34
8	5	1·5	21·5	0·0094	0·0054	−40
9	6	0·6	20·2	0·0086	0·0041	−45

of it due to direct sunlight becomes more and more obliquely incident, so that the leaf-excess-temperature falls from 5°·6 to 0°·6 C., bringing a steady fall of assimilation. The bath is, however, kept up to about 19° C. by circulating water, so that the leaf-temperature cannot fall as fast as the illumination, and the assimilation shows a larger and larger deficit below the maximum. The whole experiment exhibits the assimilation as a function of the illumination, and not limited by the temperature.

Experiment VII, on the contrary, shows the assimilation as a function of the temperature; it was a brighter day, and the leaf-chamber was continually adjusted normal to the sun's rays.

Experiment VII (July 23, 1904).—Cherry-laurel; Weight, 1·72 grammes; Area, 52·5 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 2·1$ per cent. (average).

Leaf-chamber adjusted Normal to Sun throughout Day.

Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
A.M. 8.20—9.20	—	° —	° —	Preliminary		
9.20—9.50	Fairly bright sun ...	19·3	25·0	0·0165	0·0054	0·0117
9.50—10.20	10.3—10.9, heavy cloud; 10.12, bright sun; then dull	18·5 18·9 18·8	21·2 21·9 21·3	0·0172	0·0045	0·0095
10.20—10.50	10.27, faint sun; 10.36, bright sun; 10.42, brilliant sun	19·0 19·6 20·1	22·2 27·2 29·2	0·0181	0·0052	0·0115
10.50—11.20	11, bright sun; 11.15, cloudy	20·1 19·6	30·1 23·7	0·0180	0·0052	0·0115
11.20—11.50	Cloudy..................	19·1	22·3	0·0181	0·0042	0·0091
11.50 A.M.—12.20 P.M.	Heavy clouds	19·2	21·3	0·0146	0·0041	0·0088
P.M. 12.20—12.50	Heavy clouds	19·3	20·6	0·0184	0·0036	0·0076

The readings only last half an hour each, and the temperature and illumination were recorded every three minutes throughout the experiment, so that full data for a careful analysis are available. The following table exhibits the significant numbers and averages:—

Table II.—Experiment VII.

1. Middle hour of estimation.	2. Difference between calculated and observed temperatures, 4 *minus* 3.	3. Leaf-temperature calculated from assimilation (data on p. 413).	4. Average observed leaf-temperature.	5. Observed assimilation.	6. Assimilation maxima for observed temperature (data on p. 413).	7. Difference, 5 and 6.	8. Excess leaf-temperature over bath.
9.35	−0°·3	25°·3	25°·0	0·0117	0·0115	+ 2	5°·7
10.5	−0·2	21·6	21·4	0·0095	0·0094	+ 1	2·5
10.35	+0·9	25·0	25·9	0·0115	0·0120	− 5	5·7
11.5	+1·2	25·0	26·2	0·0115	0·0122	− 7	5·2
11.35	+1·3	21·0	22·3	0·0091	0·0099	− 8	2·9
12.5	+0·8	20·5	21·3	0·0088	0·0093	− 5	2·3
12.35	+2·5	18·1	20·6	0·0076	0·0088	−12	1·3

In the last reading the sky is heavily overcast, the excess leaf-temperature and the observed assimilation the smallest recorded. The light is obviously the limiting factor, and the assimilation well below the maximum.

In the other readings the observed assimilation is close to that calculated as maximal from the observed leaf-temperature. The table is designed to show how closely one can calculate the leaf-temperature from the observed assimilation, viz., Column 3 from Column 5, the error being given in Column 2; and also, inversely, calculate the assimilation from the temperature, viz., Column 6 from Column 4, the error being in Column 7.

The temperature error (omitting the last reading) averages only 0°·8 C., and the assimilation error 0·0005 gramme CO_2. It is to be noted that there are no "preliminaries" between the different readings, so that the effects here tend to run into one another.

The relation of temperature and assimilation is exhibited as an extraordinarily constant one when one recalls that the temperature maxima used in the calculation are derived from experiments made at a different season of different years, and with artificial light.

Section IV.—*Illumination and Temperature as "Limiting Factors" in Assimilation.*

We now pass to the consideration of some experiments which are not merely records of the hourly march of natural illumination and temperature, but in which the conditions are manipulated to bring out certain points.

In Experiment VIII the leaf was for the first three readings exposed only to the diffuse light of a brilliant cloudless day. The sun's direct rays were throughout intercepted by the shadow-board, and the readings show the slow falling-off, characteristic of the afternoon diffuse light. The temperature of the bath was 30° C., and that of the leaf would be about 32°. The absolute amounts of real assimilation in the first three readings 0·0122, 0·0118, 0·0109, are such as would be maximal for the temperatures of 26° C., 25°·5 C., 24° C.; the diffuse light is evidently, though adequate for the possible assimilation at these temperatures, not adequate for that at 32°. To provide evidence in this direction, the shadow-board was removed at 3.30, and the leaf adjusted normal to the sun's rays, when the assimilation at once went up to nearly double, *i.e.*, to 0·0200. The bath became 1° hotter and the leaf would be heated 5° to 6° C. higher still by the sun's direct radiation, so that in substantial harmony with this we find the new amount of assimilation to be that maximal for 34°·6 C., or for a degree or two higher when allowance is made for the fact that the heating has been prolonged for four hours: at this temperature the time factor is not negligible.

Experiment VIII (July 18, 1904).—Cherry-laurel; Weight, 1·98 grammes; Area, 60 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 2·4$ per cent. (average).

Exposure.	Time.	Illumination.	Temperature of bath.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
Leaf-chamber exposed to diffuse light only Shadow-board	11.30 A.M.—12.30 P.M.	Cloudless, thin haze over sun	30°·2	Preliminary		
	P.M. 12.30—1.30	”	30·1	0·0375	0·0115	0·0122
	1.30—2.30	”	30·1	0·0392	0·0111	0·0118
	2.30—3.30	”	30·1	0·0362	0·0100	0·0109
3.30 P.M., leaf exposed to sun *plus* diffuse light	3.30—4.30	”	31·1	0·0350	0·0197	0·0200

The assimilation-value in the diffuse light is here an exact measure of its intensity, as the temperature is not limiting. It is thus proved not to be very intense, which is due to the fact that the day was cloudless, and but little sun was therefore reflected by clouds into the "shade." A distinctly higher value 0·0146 was obtained in Experiment II, and even that was limited by the temperature of 29° C., while in the next experiment a very much higher value is obtained on a day with abundant white cumuli.

Experiment IX.—In this experiment with *Helianthus* the temperature of

Experiment IX (August 7, 1904).—*Helianthus tuberosus*; Weight, 1·20 grammes; Area, 53·3 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 5·8$ per cent. (average).

Exposure.	Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm².
Leaf exposed to small patch of diffuse sky-light through wooden tube	A.M. 8.30—10	Close white cumuli	29°·2 29·8	29°·5	Preliminary		
	10—10.30	Mostly cloudy ...	29·2 29·8	29·5	0·0453	0·0019	0·0053
Tube removed, shadow-board, full diffuse light	10.30 A.M.—12.30 P.M.	—	29·0	30·0			
	P.M. 12.30—1	Bright sun with cumuli	29·2	30·0 31·0	0·0503	0·0102	0·0212
Leaf adjusted normal to sunlight only; wooden tube	1—2	Bright sun, a few clouds	—	30·0 31·0			
	2—2.15	Bright sun (1 min. cloud)	—	29·0 30·0	0·0255	0·0042	0·0177
	2.15—3.2	Cloudy					
	3.2—3.42	Good sun (3 mins. cloud)	—	29·0 30·0	0·0708	0·0126	0·0195
Bath darkened...........	5.50—7.15	Dark	29·5	29·5	CO_2 free	CO_2 respired	
	7.15—8.15	,, ...	29·5	29·5	—	0·0018	
	8.15—9.15	,, ...	29·5	29·5	—	0·0020	

the leaf was directly determined thermo-electrically, and an attempt was made to keep the leaf-temperature uniform by altering the temperature of the water circulating through the bath. When the leaf has to be exposed to direct sun then the gas-supply to the water-heater is diminished, or the rate of water circulation is increased, so as to compensate for the warming up produced by direct insolation.

This was a bright day, on which the sky was more or less closely packed with brilliant white cumuli.

For the first reading the wooden tube was fitted on to the bath and the tube pointed, not towards the sun but to a part of the sky away from the sun and nearer the zenith. A comparatively small number, 0·0053, was obtained (but not so small as expected), corresponding to the patch of diffuse light that finds its way down the tube. The second reading is taken in full diffuse light behind the shadow-board, and reaches the high value of 0·0212. This, as might well be expected, represents the full effect of the light, and is not limited by the temperature; Curve A in fig. 2 shows that a very much larger assimilation would be possible at this temperature. Then several readings in direct sunlight only are taken by replacing the wooden tube and keeping it pointed to the sun. The assimilation of 0·0177 in the first of these readings is much smaller than the one in diffuse light, but subsequently a more nearly equal reading of 0·0195 (or, allowing for the three minutes of cloud, 0·0209) is obtained. This whole experiment shows how efficient total diffuse sky-light may be in relation to poor direct insolation alone.

The weather broke up at 3.42, and so two estimations of the respiration in the dark were made, and the values here obtained at 29°·5 C. are part of the data for the respiratory curve in fig. 2.

In Experiment X with *Helianthus* the leaf-temperature was, for most readings, kept much lower, and it will be seen that therefore the amounts of assimilation are limited by the temperature. Rain fell on and off throughout the day, and no sun appeared till the last reading at 3.42 P.M. The chamber faced south and 30° above the horizon throughout the day, unmoved, thus receiving a large amount of diffuse light from the sky. When the sun appeared for the last reading the shadow-board was used to intercept its rays. The whole experiment then is conducted in diffuse light of varying brightness.

For the first four readings the temperature was kept down to about 18° C., and, again, in the last two the temperature was the same. In all these readings except the first, which was low, due to the extremely overcast leaden sky, the assimilation numbers are remarkably uniform, 0·0089, 0·0090, 0·0089, 0·0089, 0·0092; while the light varied up and down, being especially brighter in

Experiment X (August 11, 1904).—*Helianthus tuberosus;* Weight, 1·20 grammes; Area, 61·2 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 4$ per cent. (average).

Leaf-chamber stationary all Day, facing South, elevated 30°.

Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
A.M. 9.30—10.30	Very overcast	° —	° —	Preliminary		—
10.30—11	"	—	17·7	0·0362	0·0034	0·0062
11—11.30	Raining, but lighter	17·0	18·0	0·0362	0·0050	0·0089
11.30—noon	Raining, lighter still	17·1	18·0	0·0377	0·0051	0·0090
P.M. noon—12.30	Raining, heavy clouds	17·0	17·7	0·0390	0·0050	0·0089
1.10—2.20	Raining	Heated 29·8				
2.20—2.50	"	29·8	30·5	0·0240	0·0087	0·0163
2.50—3.12	No rain, lighter	Cooled 16·6				
3.12—3.42	Much brighter	16·6	18·2	0·0235	0·0050	0·0089
3.42—4.12	Sun out, but leaf shaded by the shadow-board	16·6	18·4	0·0254	0·0052	0·0092

the last two readings. This can only be interpreted as being due to the fact that the assimilation is limited by the temperature, which has been kept steady throughout.

Striking confirmation of this is obtained by raising the temperature for the fifth reading. The sky was no lighter than before, but yet, on the temperature being brought up to 30·5, the assimilation at once doubled, becoming 0·0163. This number will then be the exact expression of the intensity of the illumination only, for an assimilation of at least 0·0289 is possible at this particular temperature.

Nothing could show better than this experiment the impossibility of investigating the effect of varying illumination while ignoring the leaf-temperature.

In Experiment XI with *Helianthus,* the temperature of the bath was varied up and down several times for alternate pairs of readings, and careful records of the temperatures of the leaf and of the bath were made every 10 minutes, or less, according to the variability of the insolation.

Experiment XI (July 28, 1904).—*Helianthus tuberosus;* Weight, 1·96 grammes; Area, 71·2 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 2·4$ per cent. (average) till 3.10 P.M., and 5·0 per cent. (average) after 3.10 P.M.

Leaf-chamber adjusted Normal to Sun throughout the Day.

Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
A.M. 9.15—10.40	Heavy cumuli.........	° —	° —	Preliminary		
10.40—11.10	10.48, thin cloud; 10.52, slight rain; 11, cloud haze	19·5 18·1	22·7 22·0	0·0183	0·0086	0·0131
11.10—11.40	11.10, cloud haze; 11.30, heavy cloud	18·0 17·7	20·8 20·6	0·0192	0·0072	0·0109
11.40 A.M.— 12.10 P.M.	11.40, bright sun; 12, cloud haze	Heated 26·4 27·2	35·0 31·0			
P.M. 12.10—12.40	12.10, sun; 12.25, faint sun; 12.30, faint sun	27·8 28·1 28·4	33·8 30·5 29·4	0·0191	0·0180	0·0275
12.40—1.10	12.40, sun; later overcast	28·0 27·9 28·5	32·0 29·6 28·4	0·0191	0·0117	0·0184
1.10—1.40	Bright sun; 1.30, faint sun	Cooled 18·3 17·9	24·4 21·6			
1.40—2.10	1.40, thin cloud; 1.50, dull; 2, bright sun	17·7 17·6 17·7	21·4 22·6 22·2	0·0194	[0·0110	0·0166]
2.10—2.40	2.10, faint sun; 2.30, faint sun	17·6 17·3	22·6 21·8	0·0195	0·0084	0·0128
2.40—3.10	2.44, sun; 2.52, thin cloud	Heated 23·8 27·6	29·4 28·2	0·0200		
3.10—3.40	3.12, faint sun; 3.31, light sun	27·2	28·8 29·6	0·0290*	0·0145	0·0222
3.40—4.10	—	Gas out	—	0·0350	—	—
4.10—4.40	4.30, faint sun.........	Cooled 18·9 17·6				
4.40—5.10	Thick cloud............	17·9 17·2	17·8 17·6	0·0345	0·0055	0·0084
5.10—5.40	Cloudy..................	Heated 25·6 28·6	29·2 29·0			
5.40—6.10	Cloudy........	28·7 28·4	29·4 28·6	0·0332	0·0038	0·0071

* CO_2 supply increased, previously insufficient, see third reading.

Both temperatures are plotted in fig. 5, and the difference between them gives a measure of the intensity of the solar radiation at any time.

Several errors and mishaps vitiate the middle part of this experiment, but it is, nevertheless, valuable; there is a very instructive difference between the effect of raising the temperature near noon, when the light is strong and the temperature limiting, and of again raising it late in the afternoon, when the light itself is limiting.

In the first two readings the bath-temperature is about 18° C., and the leaf-temperature 4° to 2°·5 higher, giving, firstly, 0·0131 at the average temperature of 22°·2 C., and secondly 0·0109 at 20°·7 C. The day is now bright, though not clear of clouds. The light is much in excess of the above values, and they are taken as maximal for their respective temperatures (see fig. 2). They fall into one curve with the value of 0·0090 at 18° C. obtained in Experiment X.

After these two readings hotter water was circulated through the bath to give a temperature of about 27° C. The sun was irregular, and its outbursts show clearly on the temperature records, the leaf responding more acutely than the bath. The value 0·0275 was recorded at 31°·2 C. This large number is not, however, maximal, though the light is bright enough, and it was undoubtedly limited by a quite unusual factor—the CO_2 supply. Not anticipating such vigorous assimilation, we had only provided 0·0191 gramme CO_2 per half-hour, and the table shows that the leaf had absorbed as much as 0·0180. There is thus quite an inadequate margin of supply, and presently the CO_2 was increased. The fourth reading falls into quite dull illumination, as the closeness of the two temperature curves shows, and is no doubt limited by the light, giving only 0·0184 at 30° C. (*cf.* fig. 5).

After these readings the bath was cooled again. The fifth reading must be due to an undetected error of some sort, for it appears to be much *above* the maximum for the recorded temperature, while the sixth is normal again, and just about the same as the first, in temperature and assimilation, though light is much brighter and temperature-difference is large. Then a second time the bath is heated up and the assimilation rises to 0·0222 at 29° C. The next reading is spoilt by a drop in the temperature due to accidental extinction of the gas of the water-heater.

Once more the bath is cooled down, and as the sky is thickly clouded the temperature-difference is now very small. The assimilation, 0·0084, is a little below the maximum for 17·7, and the light should be the limiting factor. This is made evident by heating up again for the last time, when in spite of a return to the temperatures about 29° C., there is, in contrast, no rise of assimilation, but a further fall with the decreasing light, which also reduces considerably the temperature-difference.

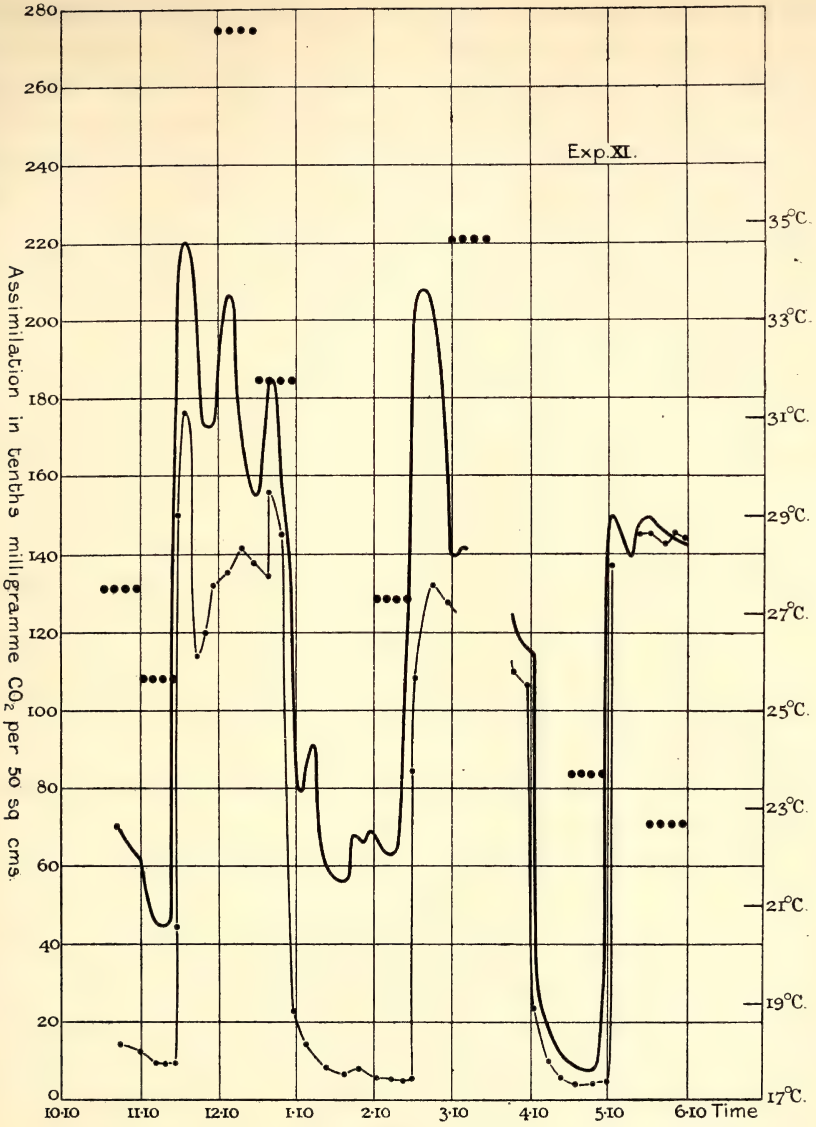


Fig. 5.

Explanation as in fig. 4, p. 424.

The contrast between the effect of the last heating and the effect of the first two heatings is very marked in the figure.

Experiment XII is an interesting example of the extremely rare case of a leaf that has temperature-maxima departing conspicuously from the standard maxima. It was cut from a shrub of cherry-laurel growing in a pot, not from the garden shrub used for all the other published experiments, but other leaves of this pot-plant gave normal numbers. Its behaviour is governed by the same principles as that of normal leaves, but it is more feeble throughout; it is instructive to note that its maxima at 26° C. and 32° C., viz., 0·0103 and 0·0139, bear such a ratio to one another as to indicate that this leaf has a somewhat smaller coefficient of temperature acceleration than a normal leaf. If so, the feeble leaf would differ from normal cherry-laurel leaves in just the same way as the latter differ from leaves of *Helianthus* (*cf.* Section VI).

Experiment XII (August 1, 1904).—Cherry-laurel; Weight, 1·79 grammes ?; Area, 60 sq. cm.; Current Rate, 800 c.c.; CO_2 = 2·1 per cent. (average).

Exposure.	Time.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
Leaf exposed to whole diffuse light *plus* half sunlight (angle incid. = 60°)	A.M. 9—10	26°·2	Prelim	inary	
	10—10.30	26·0	0·0318	0·0053	0·0103
	10.30—11	Heated 32·0			
	11—11.30	32·0	0·0308	0·0063	0·0131
Whole diffuse light *plus* whole sunlight (angle incid. = 0°)	11.30—noon	32·0			
	noon—12.30 P.M.	32·0	0·0320	0·0068	0·0139
Whole diffuse light *plus* half sunlight (angle incid. = 60°)	P.M. 12.30—1	32·0			
	1—1.30	32·0	0·0350	0·0064	0·0133

This leaf was so placed at first that the sun's rays fell on it obliquely with an angle of incidence = 60°, *i.e.*, the sunlight was of only half its full intensity; the collective diffuse light fell on it unimpeded. When the leaf-temperature was 26° C. the assimilation was only 0·0103 instead of 0·0121 (the normal maximal value for 26° C.). The day was very bright and sunny, and this clearly is the maximum for the temperature. That this is so was proved in the second reading when, with the same intensity of light (the chamber being continually readjusted to the angle of 60°), raising the temperature of the bath sent the assimilation up 25 per cent.

At about 32° C. the value obtained is 0·0131, very nearly or approaching maximal. Next the light is in its turn increased by adjusting the chamber normal to the sun's rays so as to double their intensity, and the temperature is kept down still to 32° C. There is only a small increase of assimilation and that may possibly only be due to unrecorded increase of temperature.

This failure to show a marked increase with marked increase of light proves that the assimilation is limited by the temperature of 32° C. For the last reading the light is diminished again, while the temperature is maintained and there is no serious fall in the assimilation.

The assimilation values are then throughout limited by the temperature in the same manner as with normal leaves, and we have in one experiment the two instructive cases of (1) increase of light with stationary (limiting) temperature not causing increase of assimilation, and (2) increase of temperature with stationary (non-limiting) light causing increase of assimilation.

Section V.—*The Photosynthetic Value of Full Insolation.*

In this section we propose to give further precision to our knowledge of the relation of temperature and illumination to assimilation. We propose to ascertain what amounts of illumination correspond to certain assimilation maxima, in other words to measure the amount of assimilation corresponding to definite intensities of insolation.

The procedure consists in experimenting with exactly known fractions of full midday sunlight.

Various methods have been used for reducing the intensity of sunlight to a known extent. We made a few experiments with a method based on varying obliquity of incidence of the light and also with one employing different positions in the diverging cone of light from a condenser-lens. These could not be accurately applied without elaborate adaptation. The "photometer-wheel"* of rotating sectors can be used to transmit any desired fraction of total incident light, but seems inadmissible for such work as ours because it fractions the light *in time* and not *in intensity.* If set to half opacity it allows the full intensity of light to pass for half the time, and when rapidly rotating this gives *to the human eye* an impression of steady light of half intensity. For an assimilating leaf it would give only illusory results except when the light was a "limiting factor." Thus if, in a particular case, a leaf were limited by its temperature to using just 50 per cent. of the total sunlight, then halving the actual intensity should not diminish the assimilation (provided of course that the temperature were kept up), while halving this total illumination in time should reduce the assimilation to one-half.†

Finally, we made use of the ideally simple method of employing fractioning screens made of thin metal plates perforated with holes.‡ These were placed across the mouth of the 4-foot tube directed towards the sun, that had been used to cut off the diffuse sky-light from the leaf. Different specimens of commercial wire gauze and perforated zinc were employed, and the area of their perforations and the distances of these apart were measured with a microscope. The total percentage of opening was thus obtained and the percentage of sunlight transmitted would be the same. At a distance of 4 feet these screens, the holes in which were less than 3 mm. in diameter, cast a perfectly uniform field of light. Since the sun's disk subtends a sensible angle (0° 32′) at the earth's surface, a diverging cone of rays will be transmitted through each perforation. These cones soon interpenetrate and give a more and more uniformly lighted field the further the illuminated object is from the screen. With perforations 3 mm. in diameter the fully insolated points die out at 33 cm. from the screen, and at less than four times this distance no lack of uniformity of illumination can be detected by the eye.

---

* See Abney, 'Phil. Trans.,' 1887, and Langley, 'Phil. Mag.,' 1889, vol. 27.

† See, however, Note B, p. 459.

‡ Our attention was directed to these by Professor Liveing.

For the fractions obtained to be of any precise significance, the initial sunlight must be of approximately the same intensity in the various cases, so that only unclouded weather within the hours close to the middle of the day is available. We have consequently had to make a number of unsuccessful attempts before the required data could be collected. In some cases a reading has had to be interrupted in the middle while a single cloud drifted up across the sun, and has been taken up again when the cloud has gone, allowing, of course, sufficient interval for the passing away of the effect of the momentarily diminished assimilation on the CO_2-content of the current. When a reading has been obtained in this way it will be stated.

We propose to determine first what fraction of full sunlight must be incident upon a leaf of cherry-laurel to enable it to carry out its maximal assimilation at 29°·5.

Experiment XIII, August 9.—The chamber was in the first instance faced to the sun, and received full intense sunlight together with the diffuse light of a cloudless sky. In the first reading, 9.30 to 10 A.M., the assimilation is 0·0152 gramme per hour, at an average leaf-temperature of 29°·7 C. The temperature of the leaf was taken a number of times during this and subsequent readings, and was always kept adjusted close to the average given, by altering the temperature of the water circulation. With this intense illumination the assimilation must be maximal, and the value is just above the standard maximum for 29°·7, *i.e.*, 0·0148, due to this being an early reading.

For the second reading the wooden tube is put on to the chamber, so that the leaf receives only direct sunshine without the general diffuse light. The assimilation is, however, not lowered (but, as it happens, a trifle larger) by the

Experiment XIII (August 9, 1904).—Cherry-laurel; Weight, 1·78 grammes; Area, 56·2 sq. cm.; Current Rate, 800 c.c.; CO_2 = 5·8 per cent. (average).

Exposure.	Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
Leaf normal to sun *plus* diffuse light	A.M. 9—9.30	Intense sun, cloudless	20°·9	28°·5	Preliminary		
	9.30—10	" "	22·1	29·7	0·0437	0·0073	0·0152
Leaf normal to sun only, with tube	10—10.18	" "	22·3	29·2			
	10.18—10.48	" "	22·3	29·5	0·0450	0·0074	0·0154
Leaf in diffuse light only; shadow-board	10.48—11.48	Clouding over.					
	11.48 A.M—12.20 P.M.	Very little sun, heavy white cumuli	—	29·1			
	P.M. 12.20—12 50	" "	—	29·1	0·0487	0·0065	0·0136
Chamber darkened for respiration estimations	2.45—4	Darkened	30·0	30·0	—	Respiration	
	4—5	"	30·0	30·0	—	0·0021	
	5—6	"	30·0	30·0	—	0·0024	

diminution of light, because it is in both cases limited by the temperature to the maximal value.

As the sky then began to cloud up it was impossible to employ the fractioning screen, and a reading was therefore taken of the effect of diffuse light only, removing the sun-tube, and putting up the shadow-board. With this illumination we got an assimilation of 0·0136 at 29°·1 C., which is a trifle below the standard maximum for the temperature, 0·0143, which may well be due to the decline after three hours' maximal assimilation at this fairly high temperature.

These values are plotted with crosses in fig. 6, where the lower dotted line represents the curve of assimilation falling off with the time-factor from the hypothetical initial value of 0·0167, through the standard value of 0·0148 (after about two hours), to the observed value of 0·0136 after three and a half hours.

Apart from the time factor it is obvious that all three kinds of illumination —diffuse light alone, direct sun alone, and direct sun *plus* diffuse light—are producing practically the same amount of assimilation, not, of course, because they are equal in intensity, but because the assimilation is limited by the temperature, all the lights being in excess.

Having by this experiment got full evidence as to the magnitude of the assimilation maximum at 29°·5 C., the next experiment was planned for a fraction of the sunlight. A piece of gauze was employed that transmitted 0·62 of the whole incident light.

In Experiment XIV the leaf was set up at 9.15; the day was cloudless, but with a thin haze. As the haze obstinately remained, the chamber was darkened for two hours. Then at 11.25, the haze being nearly gone, the wooden tube was put on with the 0·62 screen, and this kept pointed to the sun. An estimation at noon gave 0·0154, just the theoretical value as shown in fig. 6, and maximal for the leaf-temperature, showing that even 0·62 sunlight is more than sufficient for maximal assimilation at 29°·5 C.

Heavy clouds came up soon after this reading, and the experiment was abandoned, and the next one was made with a piece of perforated zinc with much smaller holes, and transmitting 0·28 sunlight.

In Experiment XV a leaf was set up by 9 A.M.; the day was brilliantly sunny, with large, but very remote, cumuli drifting slowly across the sky. At 10.14, after a long cloudless spell, it seemed that an estimation might be started, but this had to be interrupted in the middle for the passage of one big cloud across the sun. At the moment of eclipse the current was shifted by hand back into the preliminary tube, and kept there for 20 minutes after the cloud had passed, and then it was returned to the estimation tube. At

Experiment XIV (August 13, 1904).—Cherry-laurel; Weight, 1·48 grammes; Area, 49·0 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 5{\cdot}8$ per cent.

Exposure.	Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
Leaf darkened	A.M. 9.15—11.25	Hazy	°	°			
Leaf exposed to 0·62 sunlight with tube	11.25—11.50	Haze melting	—	29·0	Preliminary		
	11.50 A.M.—12.20 P.M.	Cloudless sunshine...	27·0	28·7—30·4	0·0452	0·0065	0·0154

Experiment XV (August 16, 1904).—Cherry-laurel; Weight, 1·78 grammes, Area, 56·0 sq. cm.; Current Rate, 800 c.c.; $CO_2 = 6$ per cent. (average).

Leaf exposed to 0·28 sunlight with tube	A.M. 9—10.14	Very bright sun, occasional clouds	28·5	29·7	Preliminary		
	10.14—11.8 32 mins. sun	,, ,,	28·3	29·4	0·0492	0·0058	0·0116
Leaf exposed to full sunlight with tube	11.8—11.45	Very bright sun, no clouds	22·1	29·8			
	11.45 A.M.—12.15 P.M.	,, ,,	22·1	29·6	0·0467	0·0068	0·0141

11.8 a second cloud came up, and the reading was ended, having consisted in all of 32 minutes of brilliant sunshine. The assimilation per hour with 0·28 sunlight was 0·0116 for 29°·4 C., distinctly less than 0·0147, the maximum which the temperature should allow. The metal screen was then removed, and a reading in full sunshine obtained, which gave the value of 0·0141 at 29°·6, which is just the value we should expect after three hours' high assimilation.

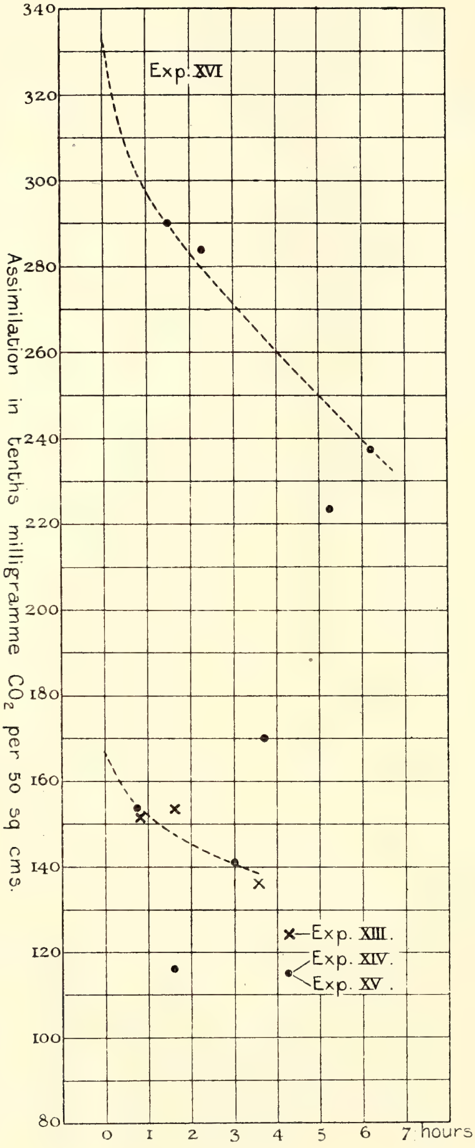


Fig. 6.

These data are plotted in fig. 6, where the line from 0·0167 to 0·0140 represents the fall of the assimilation maxima at 29°·5 due to the time-factor.

We see from this, that at 10.30 A.M., $1\frac{1}{2}$ hours *ab initio*, we should expect a value of 0·0148. With 0·28 sunlight at this time in Experiment XV we get a value of 0·0116—*i.e.*, 0·28 sunlight gives 116/148 of maximal assimilation at 29°·5. Therefore, to give the whole maximal assimilation ($1\frac{1}{2}$ hours *ab initio*), there will be required 0·36 ($= \frac{148}{116} \times 0{\cdot}28$) sunlight.

One might make an apparently more direct and absolute statement to the effect that *the photosynthetic energy in* 0·28 *sunlight is sufficient to reduce* 0·0116 *gramme* CO_2, but we shall see presently reason for regarding the statement which takes notice of the time-factor as a more critical and precise one.

Before going further into this matter, let us take a similar experiment with a leaf of *Helianthus*.

After several failures, in Experiment XVI we succeeded in getting all our data into one experiment.

The leaf was set up at 8 A.M., exposed to full intense sunlight *plus* diffuse light, and the assimilation value of 0·0290 was obtained at about 30° C. This we regard as obviously maximal for the temperature. To make certain of this, a second estimation was made with direct sunlight only, the diffuse light being cut out by the wooden tube. Again, in spite of the diminution of light, about the same assimilation, 0·0284, is obtained, and this point is proved. Then the diffuse light alone is employed (and, indeed, the sky had clouded up), giving a value of 0·0170, which is not nearly maximal for the temperature of 29°·5, but only about $\frac{3}{5}$ of the maximal amount. On the clouds clearing right away an estimation was made with 0·62 sunlight at 30° C., and this gave a value of 0·0224, large, but not maximal as was the case with cherry-laurel in this intensity of light. To conclude this satisfactory series, an estimation was again made in full sunlight, *plus* diffuse light, to get a maximal reading (one cloud came up at 2.6 P.M., and the current was shifted back to the preliminary tube till 2.29 P.M.). The value obtained was 0·0238, showing the usual effect of the time-factor, and falling off from the early value of 0·0290.

Connecting up (in fig. 6) the maximal values by a line, we find that at 1.15 the value would have been 0·0248. So at that hour 0·62 sunlight gave 224/248 of maximal assimilation at about 29°·5 (5 hours *ab initio*); therefore, for the whole maximal assimilation, 0·69 sunlight is required.

The absolute form of statement would be that 0·62 sunlight can reduce 0·0224 gramme CO_2.

Experiment XVI (August 8, 1904).—*Helianthus tuberosus*; Weight, 1·30 grammes; Area, 59·0 sq. cm.; Current Rate, 800 c.c.; CO_2 = 6·3 per cent. (average).

Exposure.	Time.	Illumination.	Temperature of bath.	Temperature of leaf.	CO_2 supplied.	CO_2 absorbed by leaf.	Real assimilation per hour per 50 cm^2.
Leaf exposed to full sunlight *plus* diffuse light	A.M. 8·5—9.5	Brilliant sun	24°·8	29°·6	Preliminary		
	9.5—9.35	,, ...	24·8	29·3—31·0	0·0469	0·0159	0·0290
Whole sunlight only; wooden tube	9.35—10.3	,, ...	23·5	29·0			
	10.3—10.33	,, ...	23·5	29·2	0·0509	0·0156	0·0284
Diffuse light only; shadow-board	10.33—11.7	Cloudy	—	29·0			
	11.7—11.30	Dull	28·5	29·5			
	11.30—noon	,, ...	28·5	29·5	—	0·0089	0·0170
Leaf exposed to 0·62 sunlight; wooden tube with wire gauze	P.M. noon—1.6	Clouds; brilliant sun after 12·55	27·6	30·0			
	1.6—1.36	Brilliant sun; no clouds	27·6	30·0	0·0514	0·0120	0·0224
Full sunlight *plus* diffuse light	1.36—1.55	Bright sun	—	29·0			
	1.55—2.48 (30 mins. sun)	,, (one big cloud)	—	29·5	0·0550	0·0130	0·0238
Darkened	4—6	Dark	19·0	19·0	—	Respiration 0·0007½	

Let us now compare the results with these two very different types of leaf:—

Cherry-laurel... 0·28 sunlight gives 0·0116. ∴ full sun = 0·0414
Helianthus...... 0·62 sunlight gives 0·0224. ∴ full sun = 0·0361

The sun with the cherry-laurel was 1½ hours before noon, and with *Helianthus* was 1¼ hours after noon, so no correction is to be applied on that account.*

The larger value was obtained on August 16, and the smaller one on August 8, when the brightness of the sun at noon is about 3 per cent. greater, so that correction for the greater air mass traversed by the rays of the lower sun of the later date would tend to bring the numbers further apart by this amount.

Apart from any correction there is a fair agreement between these values as indicating that the full sun, about noon, about the middle of August, radiates enough light on to 50 sq. cm. of leaf surface, normally exposed, to reduce 0·04 gramme CO_2 per hour. At the summer solstice the value should be about 6 per cent. larger.

There is, however, a biological correction, legitimately to be applied to the two values, which brings them much closer together. This turns on the fact that the value for cherry-laurel was determined 1½ hours after starting assimilation, while for *Helianthus* it was 5 hours after starting; the "time-factors" are different.

There is evidence, to be considered in a separate paper, that the time-factor at high temperatures is not of the nature of a limiting factor, but involves a falling-off of the efficiency of the chloroplast machinery of such a nature that it takes, to put it crudely, continually more energy to reduce a given amount of CO_2. The chloroplast will, at any time, require a fixed amount of light-energy, but will reduce less and less CO_2; also, with any less amount of energy, there will be a proportional decrease in the amount of CO_2 reduced. If, then, at any one moment at the temperature of 29°·5 with cherry-laurel, 0·36 light gives maximal assimilation, then, according to this interpretation, it would give it also at any other moment.

So, also, with *Helianthus*, 0·69 light will always give just the maximal assimilation at this same temperature.

What, then, is the ratio of the maximal assimilation at about 29°·5 C. for these two leaves? Fig. 6 shows that, for those times at which there is evidence as to both, the value for *Helianthus* happens to be 1·95 times that for cherry-laurel. We should expect *Helianthus* to require 0·71 sun if cherry-laurel requires 0·36, and what it does require is 0·69 of sunlight 8 days nearer the summer solstice, when the sun is 3 per cent. brighter. This correction for the time-factor then brings the estimations into extraordinary close harmony, some of which is no doubt accidental, and may vanish with further experiments.

---

* Crova has shown that after noon the more abundant water vapour renders the atmosphere less transparent to heat radiations than before noon, but there is no evidence that this holds for light.

If the hypothetical initial values of the temperature maxima at 29°·5 C. really hold for any appreciable time, and the time-factor has the significance here attributed to it, then the full sunlight will have a higher absolute photosynthetic value than the observed value. For, *initially*:—

	0·36 sun, on August 16, would give……	0·0167
and	0·69 sun, on August 8, would give……	0·0325

if these are the initial maxima for cherry-laurel and *Helianthus* respectively at this temperature.

On this basis—

Full sun, on August 16 = 0·0464
Full sun, on August 8 = 0·0471

which values have about the right proportion for the brightness of the noon sun at these two dates.

The photosynthetic value of the full noontide sun at the summer solstice would then be about 0·050 gramme CO_2 per 50 sq. cm. of leaf surface normally exposed at this latitude.

These refinements of correction have no great significance till more estimations have been made, but somewhere between 0·05 and the uncorrected value of 0·04 the actual value will lie.

Section VI.—*The Specific Assimilational Characteristics of Diverse Leaves.*

This section will be devoted to some physiological considerations suggested by the differences in functional activity of the two types of leaf with which we have worked.

For one thing we find that the two leaves do not have the same assimilation-maxima at identical temperatures. This in itself does not greatly surprise, and one might expect that the "less active" leaf of cherry-laurel would perhaps reduce less CO_2 for the same amount of energy—that there would be different specific economic coefficients of chloroplast activity for the two leaves. In that case there would possibly be a constant ratio shown between their curves of assimilation-maxima. Inspection of fig. 2 shows that this is not so. The maxima are, at 18° C., in the ratio of 1 : 1·2, and at 29°·5 C. as

1:1·95. We have, however, proved in the last section that at this higher temperature *Helianthus* requires twice as much light to reach its maximum as does cherry-laurel.

This suggests that the same amount of CO_2 is reduced in both plants by the same intensity of light. At 18° C. *Helianthus* would then require only 1·2 times as much light as cherry-laurel for its maximal activity. The limitations set upon the *activity* of leaves by various temperatures would then be secondary in nature, and be merely superposed upon a primary and uniform relation between energy absorbed and work done.

The only observations that militate against this uniform relation are those dealing with the decline of assimilatory activity with continued exposure to high temperatures. These effects of the "time-factor" appear not to be such as a limiting factor would produce, but rather to indicate a smaller economic coefficient of activity.

This evidence must be postponed for the present, but we propose to give now experiments to prove that at temperatures not involving the time-factor, equal intensities of light produce the same amount of reduction of CO_2 even with very diversified types of leaf.

These experiments were carried out with artificial light (Keith high-pressure incandescent gas light) just in the same way as the experiments in "Assim. and Resp. III."

The leaf-chamber was contained in a water-bath at a uniform temperature of 25° C. The source of light was removed to such a distance that it only caused an assimilation of about 0·0085 gramme CO_2 per hour per 50 sq. cm. leaf-area. It was thus well below the assimilation-maximum for the temperature, which is, with cherry-laurel, 0·0114. This, of course, is of fundamental importance, and ensures that the amount of assimilation will really be a measure of the light-intensity.

The light used has an arbitrary intensity depending on its distance from the bath; its intensity was not determined in any optical unit, but is expressed in terms of its reducing power for CO_2 with a cherry-laurel leaf.

We first compared a leaf of *Tropæolum* with one of cherry-laurel. The experiments were as follows:—

Experiment XVII.—*Tropæolum* (October 8, 1904). Weight of leaf, 1·25 grammes; Area, 53·2 sq. cm.

Time.	CO_2 supplied.	CO_2 absorbed.
10.45—noon ……	Prelim	inary
12—1 ………	0·0283	0·0086
1—2 ………	0·0289	0·0080
2—3 ………	0·0295	0·0078

Mean apparent assimilation ………………………	0·0081
Respiration, 7.30—11.30, 0·0028 in four hours …	0·0007
Real assimilation per leaf ………………………	0·0088
” ” per 50 sq. cm. …………………	0·0083

Experiment XVIII.—Cherry-laurel (October 10, 1904). Weight of leaf, 1·90 grammes; Area, 54·3 sq. cm.

Time.	CO_2 supplied.	CO_2 absorbed.
10.45—noon ……	Prelim	inary
12—1 ………	0·0334	0·0080
1—2 ………	0·0344	0·0082
2—3 ………	0·0348	0·0082

Mean apparent assimilation ………………………	0·0081
Respiration, 7.30—11.30, 0·0049 in four hours …	0·0012
Real assimilation per leaf ………………………	0·0093
” ” per 50 sq. cm. …………………	0·0085

Then, at another time, we compared the leaves of *Bomarea* and *Aponogeton* with cherry-laurel, using approximately the same position of the light.

Experiment XIX.—Cherry-laurel (December 7, 1904). Weight of leaf, 2·15 grammes; Area, 62 sq. cm.

Time.	CO_2 supplied.	CO_2 absorbed.
11.15—noon	Prelim	inary
12—1	0·0378	0·0095
1—2	0·0393	0·0102
2—3	0·0384	0·0104
3—4	0·0371	0·0094

Mean apparent assimilation	0·0099
Respiration, 5.30—11.30, 0·0066 in six hours ...	0·0011
Real assimilation per leaf............................	0·0110
„ „ per 50 sq. cm.	0·0089

Experiment XX.—*Bomarea* (December 8, 1904.) Weight of leaf, 1·40 grammes; Area, 51·7 sq. cm.

Time.	CO_2 supplied.	CO_2 absorbed.
11.15—noon	Prelim	inary
12—1	0·0360	0·0092
1—2	0·0336	0·0083
2—3	0·0352	0·0081
3—4	0·0352	0·0076

Mean apparent assimilation	0·0083
Respiration, 6—12 night, 0·0027 in six hours ...	0·0005
Real assimilation per leaf............................	0·0088
„ „ per 50 sq. cm.	0·0085

Experiment XXI.—*Aponogeton* (December 10, 1904). Weight of leaf, 1·60 grammes; Area, 39·5 sq. cm.

Time.	CO_2 supplied.	CO_2 absorbed.
12.30—1.15......	Prelim	inary
1.15—2.15......	0·0400	0·0072
2.15—3.15......	0·0410	0·0066
4.45—5.45......	0·0418	0·0066

Mean apparent assimilation	0·0068
Respiration, 7.20—11.20, 0·0015, in four hours...	0·0004
Real assimilation per leaf............................	0·0072
„ „ per 50 sq. cm.	0·0091

The leaf of *Aponogeton*, which normally floats on water, was here simply stood up with its cut stalk dipping in the water at the bottom of the chamber. The air in the chamber was quite damp, and the leaf showed no signs of wilting at the end of the experiment. *Bomarea* is a climbing monocotyledon with its morphologically upper leaf-surface downwards.

The four other leaves examined, then, agree with our standard within 5 per cent., and the diversity of type of these five seems to us to be wide enough to prove that *leaves in general have the same coefficient of economy in the photosynthetic process.*

There is, then, no difference in leaves in this direction, and it would appear from fig. 2 that even their temperature-maxima would be the same at very low temperatures. The fundamental existing specific differences would seem to lie in their different coefficients of acceleration of activity with increase of temperature. For a rise of 10°, the increase with cherry-laurel is 2·1 [0·0038 at 9° and 0·0080 at 19° C.], while with *Helianthus* it is certainly bigger, perhaps 2·5, but we have not exact data yet for giving the coefficient a precise value.

Perhaps this specific difference of the coefficient of temperature acceleration holds with growth and other metabolic processes, and a high coefficient might be a general characteristic of those plants which are recognised to be very "active" in vegetation.

The general views expressed in this paper involve the assumption that *with all intensities of light the amount of assimilation is proportional to the intensity of the light* unless some secondary or limiting factor is at work. Timiriazeff* has recently expressed the view that for plants (in general) there is a maximum of assimilation corresponding to half the intensity of direct sunlight, and that with higher intensities no further increase of assimilation takes place.

We feel convinced that this result is due to the neglect of some limiting factor, probably the temperature, the effect of which has been ignored by nearly every investigator of these questions. By selecting an appropriate temperature one can get a maximum of the kind described by Timiriazeff at any desired low fraction of sunlight for any given leaf.

There is certainly no *general* value for the fraction of sunlight utilised by leaves, either at a given temperature, or at the highest functional temperature, but, on the contrary, it is just on this point (being a consequence of the combined principles of uniform economic coefficient and varied temperature-acceleration coefficients) that leaves differ specifically one from another.

---

* Timiriazeff, 'Roy. Soc. Proc.,' vol. 72, p. 451, 1903.

It follows that there is *no optimum* of light-intensity for assimilation, even for specific leaves, still less generally.

Section VII.—*The Limitation of Assimilation by the Natural Environment.*

The relation of the respective intensities of sunlight, diffuse sky-light, and total light become of interest in connection with our estimations, and with the general question of the available illumination for "sun plants" and "shade plants." The relation of these intensities has been calculated for a cloudless sky by Clausius.* The calculations are based on a summing up of the various orders of reflections of that part of the sunlight which is scattered in passing through the atmosphere.

Assuming the light that would reach a horizontal surface on the earth from a vertical sun to be unity, if the atmosphere were absolutely clear and did not scatter any of it, Clausius calculated that with the sun at an altitude of 60°, the direct sunlight would be 0·621 and the diffuse light 0·176, while at an altitude of 30°, the former would be 0·281 and the latter 0·138, and at an altitude of 10°, the former 0·033 and the latter 0·067.

Thus, as the sun sinks the ratio of the diffuse light to the sun-light increases, being about 1 to 2 at 30°.

Several observers have made direct observations on these points, using the darkening of photographic sensitised papers as a measure of light intensity. This only gives a measure of the more refrangible rays, and is generally spoken of as the "chemical intensity" of the radiation.

Brennand† made measurements in the cloudless sky of India and exposed his paper (1) at right angles to the sun's rays alone in a dark chamber; (2) in shadow of a stick to sky alone; and (3) to sun and sky together.

With the sun below the altitude of 13°,‡ the whole diffuse light is more active than direct sunlight alone. Above 13° he found the following values as means of a large number of observations:—

Sun's altitude.	Sunlight.	Sky-light.	Total light.
13°	0·0377	0·0376	0·0782
30	0·1070	0·0628	0·1698
45	0·1429	0·0700	0·2128
60	0·1620	0·0727	0·2347
90§	0·1751	0·0743	0·2404

---

* Clausius, 'Poggendorff Annalen,' vol. 72, 1847.

† Brennand, 'Roy. Soc. Proc.,' vol. 49, 1891.

‡ Roscoe found the altitude for equality to be 19°.

§ The values for 90° were obtained by calculation.

Here we note that sky-light increases its ratio to sunlight as the altitude decreases, being just half at 45°.

For a *horizontal* surface on the earth Brennand calculates from the above data :—

Sun's altitude.	Sunlight.	Diffuse light.	Total light.
15°	0·012	0·051	0·063
45	0·069	0·083	0·152
60	0·140	0·089	0·229

These numbers show how large a part diffuse light plays in the total illumination when the sun is low.

Roscoe* has made a large number of measurements of "chemical intensity" of radiation by the photographic method worked out by Bunsen and himself in 1862. He finds that the "chemical action" (*i.e.*, that on sensitised silver paper) of the sun depends only on its altitude independently of the hour of day or the latitude.

The presence of clouds causes large departures from the values calculated or found for cloudless skies; the "clouds act as mighty reflectors of light" and "the presence of a thin film of cloud may enormously increase the *total* chemical intensity."

The rays that act on silver salts do not, however, play the chief part in assimilation. Objective measurements of the intensity of action of the less refrangible rays of natural light have not, so far, been carried out. Possibly, the activity of the living chloroplast may be yet used for this purpose.

Measurements of the intensity of total daylight on a horizontal plate have been made daily at noon for some years by L. Weber.† He measured the intensity of the red rays (about $\lambda = 630$) and the green rays (about $\lambda = 541$) of the total daylight, comparing them photometrically by eye with the same rays in a standard artificial light. He finds that the ratio of red to green varies in the daylight independently of its intensity.

The highest value observed in three years for the total daylight at noon was on July 5, viz., 154,300, and the minimum on December 12, viz., 655, the total mean of the three years being 36,185 metre candles.

The amount of the total daylight on any given day that is attributable to diffuse sky-light was arrived at by deducting the *calculated* brightness of the sun's direct light at noon on that day. In this way it is shown that on some days, with the suitable arrangement of clouds, even at noon the total light may be as much as three or four times that of the sun alone, and that too, in summer.

---

* Roscoe, 'Phil. Trans.,' 1863, 1865, 1867, 1870; and 'Roy. Soc. Proc.,' vol. 15, 1866.

† L. Weber, 'Meteorol. Zeitschrift,' vol. 2, 1885; and 'Schriften Naturw. Vereins, Schleswig-Holstein,' vol. 10, 1895.

As regards red rays, the noon-total-daylight curve for the year agrees very closely with that calculated for red rays in clear sun alone, so that the clouds appear to reflect upon the earth just about as much red light as they stop out of the direct sunlight. This would tend to discount the influence of sunlight in a process such as assimilation.

As regards green constituent rays it is different, and the clouds in general reflect much more than they stop directly out of the sun's rays. A few observations made concurrently on the "chemical intensity" of the action of total daylight at noon on sensitised paper seemed to show that the actinic rays are proportional to the red, and about 25 times as intense (in ratio that is, to the relative abundance of these two kinds of rays in a normal candle).

The whole trend of these observations is to increase our *a priori* estimate of the assimilatory value of diffuse light as compared with direct sunlight.

This also has been the outcome of the measurements recorded in this paper. The highest assimilatory value of diffuse light recorded is 0·0192 gramme CO_2 in Experiment IX, and this is equal to all the assimilation that is possible at about 27° C. with *Helianthus*, or at 34° C. (31° initially) with cherry-laurel.

The intensity of the blue rays will not always serve as a measure of the intensity of the red rays, particularly so when the sun is low in the heavens. Abney* has measured the brightness of the various parts of the solar spectrum with different altitudes of the sun. The smaller the altitude, the longer is the path that the sun's rays travel through the atmosphere, and as the air is more absorbent for blue rays, so these tend to die out more and more as the sun sinks and the light becomes redder. We may quote the following data:—†

Sun's altitude	90°·0	30°·0	14°·3	7°·3	about 0°
Atmospheric mass	1·0	2·0	4·0	8·0	32·0
Red, A, $\lambda = 0·76$	0·95	0·91	0·81	0·66	0·107
Orange, D, $\lambda = 0·59$	0·87	0·75	0·57	0·32	0·001
Blue, F, $\lambda = 0·49$	0·74	0·54	0·30	0·09	0·000
Total sun brightness	0·84	0·70	0·50	0·21	0·002

In this connection it is interesting to recall how late in the evening assimilation can be detected. The rays active in photography die out rapidly towards sunset, but Experiments II, III, and IV show that the red rays persist later and that assimilation actually overbalances respiration until sunset, and continues to cause a gain of material to the leaf up to that time.

---

* Abney, 'Phil. Trans.,' 1887 and 1893.

† Hann, 'Lehrbuch der Meteorologie,' Leipzig, 1901, p. 13.

From natural illumination in relation to assimilation-intensity we may turn now to the question of natural temperature in the open air.

The temperature of a leaf in the shade will equal the air-temperature within a degree or so, and the temperature may therefore well be so low that it would prevent the light producing its full effect, and the temperature would then in Nature, as in many of our experiments, be a limiting factor.

If we assume that a cherry-laurel leaf in the shade is just about the air-temperature, our curve of assimilition-maxima in fig. 2 shows us at once to what extent the assimilation would be limited by the temperature. Let us take the particular case of August 7, when the diffuse light was sufficiently bright to give an assimilation of 0·0192 in the shade. We construct the following table of the effect at different temperatures :—

Leaf-temperature.	Assimilation possible.	Percentage of available light utilised.
18° C.	0·0075	39
22	0·0097	50
28	0·0135	70

In direct sunshine in open air the case would generally be different, and the absorption of total radiation by the leaf would so raise its temperature as to make it capable of utilising a larger part of the rays specific to photosynthesis.

We may consider further cases from our work. The cherry-laurel leaf in brilliant sunshine in the open air reaches possibly a temperature of 9° to 13° C. above the mercury thermometer in the sun, say a temperature of 39° C. for a bright hot day ; this corresponds theoretically to an initial assimilation value of 0·0352 gramme CO_2 per 50 sq. cm. per hour, which is about three-quarters of 0·047 gramme CO_2, the calculated "initial" value for full intensity of August sunshine, and distinctly less than the possible value of full sunshine *plus* general diffuse light at the summer solstice.

For *Helianthus* the temperature-coefficient is larger and the temperature maxima run up very rapidly at high temperatures. We have as yet no data for this leaf above 30° C., but continuing the curve upwards freely the theoretical initial value of 0·05 might even be attained at 38° C. It seems clear that this leaf might for a short time utilise the whole of the specific radiation in full August insolation at some temperature above 35° C. The temperature which a *Helianthus* leaf actually reaches in the sun in the open air is probably lower than with cherry-laurel by virtue of greater transpiration.

While then cherry-laurel in the sun would certainly have its assimilation limited by its temperature, *Helianthus* might not be thus limited when it attained a similar temperature. If, as seems probable, it only attains much lower temperatures, it also might be limited in sun alone, and certainly so when illuminated by direct insolation *plus* bright diffuse light.

Besides the illumination and the temperature there is a third factor which may function as a limiting factor. This is the partial pressure of CO_2 in the atmosphere surrounding the leaf. In all our experiments the air-current has been enriched with CO_2, so that only in one case, and that unintentionally, has the CO_2-supply been a limiting factor. How different is it in Nature!

All the considerations that we have adduced regarding the extreme values of assimilation with full intensity of illumination and the highest temperatures come to nought, at least, as far as their direct existential import, when we realise that nowhere in nature is there sufficient carbon-dioxide in the environment to permit of anything approaching such intensity of assimilation.

We may take it that the CO_2 in the atmosphere rarely exceeds three parts in 10,000 (except indeed in London fogs), and we have to enquire to what maximum of assimilation this will limit a leaf in the open air. We have made no experiments on this point ourselves, but the experiments of Sachs and of Horace Brown furnish us with data.

Horace Brown* has shown that a surface of caustic alkali exposed in the open air containing 3 parts in 10,000 of CO_2, absorbs per hour and per square metre 1200 c.c. of this gas when the air is still and up to 1500 c.c. if the air is agitated by wind.

Brown and Morris† have also determined the amount of CO_2 taken in by a leaf of *Helianthus* in the open air, by measuring the increase of dry weight after the method of Sachs.

On a bright warm day a cut leaf gained 1 gramme dry weight per hour per square metre and on a dull day a cut leaf gained 0·985 gramme, this gain is to be treated as if it were all carbohydrate of the cane-sugar type. Expressing the results in grammes CO_2 per 50 sq. cm. of area per hour, we get the following absorptions:—

---

* Address to the Chemical Section, British Association, 1899.

† 'Journal of the Chemical Society,' 1893, p. 604.

Caustic alkali, still air	0·0117
„ wind	0·0148
Helianthus leaf, bright day	0·0079[*]
„ dull day	0·0077

Sachs arrived at a much higher number for *Helianthus,* viz., 0·150, but this was not directly observed; it was arrived at by employing a leaf attached to its plant and making a large allowance for translocation, based on another experiment. Sachs' number probably is much too high.

From the fact that the same number was arrived at by Brown and Morris on two days, one brightly lighted and the other dull, we may conclude that the intake of CO_2 was really limited by the diffusion possibilities and not by the light or temperature.

This view is supported by the experiments in the present paper, which show that this *intake* of CO_2 can be much surpassed with moderate illumination when the CO_2-supply is not a limiting factor. Thus with an adequate pressure of CO_2 we have recorded:—

Experiment	XVI.	*Helianthus,*	sunlight only	= 0·0264[†]
„	XVI.	„	diffuse light only	= 0·0151
„	XI.	„	faint sun	= 0·0254
„	IX.	„	diffuse light only	= 0·0192

It is probable then that a leaf of *Helianthus* is in Nature limited to an absorption of 0·0077 gramme CO_2 per hour per 50 sq. cm. The value may be higher even up to the 0·0150 of Sachs, but it does not seem likely that the leaf should be as efficient as an equal area of caustic alkali, even though this particular leaf has two absorbing surfaces. The evidence, on the whole, is not in favour of the view that leaves with stomata on both surfaces are much more efficient absorbers than those with stomata on one surface only. There are probably few leaves more active in assimilation than those of *Helianthus,* *i.e.*, few offering less mechanical hindrance to the ingress of CO_2, and if the lower value of 0·0077 is too low for this plant, it may be the limit for a number of other plants.[‡]

We will endeavour then to picture the state of things that holds with a leaf limited to this intake by the low pressure of CO_2 around it.

In setting out to compare the energy available for photosynthesis with the work actually done under various environments, the fact must not be lost sight of that the leaf-cells are *respiring* in the light, and that the CO_2 that they produce has continually to be reduced by the radiant light-energy before so low a partial pressure of CO_2 is arrived at that further supplies may diffuse in from the atmosphere. The amount produced in respiration will vary with the temperature of the cells and may, at about 30° C., be equal to half the possible inflow from without.

---

[*] A leaf of Catalpa (conditions of experiment not stated) gave also 0·0078, *cf.* Horace Brown, 1899, *loc. cit.*

[†] These numbers are the *apparent* assimilation, *i.e.*, the real assimilation less the respiration.

[‡] See, however, Note C, p. 459.

The following table represents the waste of photosynthetic energy that may be considered to go on when the natural supply of CO_2 limits the intake to 0·0077, with various illumination and temperature.

The table avoids extreme intensities of illumination and deals with two that we have actually observed. The assimilation-maxima given in the third column are all observed values except the last, which is only an approximation. The energy is throughout expressed in terms of the work it can do in photosynthetic reduction, *i.e.*, in grammes CO_2 per hour per 50 sq. cm. leaf-area:—

Leaf.	Hypothetical temperature of leaf cells.	Assimilation-maximum.	CO_2 of respiration.	+	Intake of CO_2 from atmosphere.	=	Actual assimilation.	Aug. 8, 1904, noon.	
								In sun, 0·0530.§	In shade, 0·0170.
								Surplus energy.	Surplus energy.
	°C.								
Cherry-laurel	10	0·0042	0·0005	+	0·0037	=	0·0042	0·0498	0·0128
Helianthus ...	19	0·0098	0·0008	+	0·0077	=	0·0085	0·0445	0·0085
,, ...	29	0·0285*	0·0020	+	0·0077	=	0·0097	0·0443	0·0073
Cherry-laurel	37·5	0·0238†	0·0044	+	0·0077	=	0·0121	0·0409	0·0049
Helianthus ...	35	0·0400‡	0·0032	+	0·0077	=	0·0109	0·0421	0·0061

The last two columns show how large is the waste of photosynthetic energy with the limited intake of CO_2 that our atmosphere provides for.

It will be observed that raising the CO_2-content of the atmosphere from three up to six parts in 10,000 will, as Horace Brown has shown, double the intake, and this will do away with all waste of energy in the last four cases in the shade. To prevent this waste in the insolated leaf an increase of six fold, to about 18 parts in 10,000, would appear to be indicated. As a matter of fact, if this were done the temperature would then become the limiting factor and each leaf would stop at the values given in column 3. The waste would then be the difference between 0·0530 and the values in this column.

---

* Observed 1¼ hours *ab initio.*

† ,, 2 ,, ,,

‡ Approximately estimated.

§ This represents the energy available for photosynthesis in Experiment XVI; in that case (reading 4) 0·62 sun gave 0·0224 ∴ whole sun alone may be taken at 0·0360. The diffuse light (reading 3) was 0·0170; for a leaf freely insolated these two must be added together.

The leaf at 10° C. is, however, already limited by temperature, and cannot, therefore, avail itself of even half the CO_2 that might diffuse into it.

At hours remote from noon the solar radiation falling on a leaf will be much less intense, and the average for all the daylight hours of a year will be very much below 0·0530.

This consideration is of interest in relation to the theory that the reduction of carbon-dioxide by plants in the carboniferous epoch must have been much more energetic than at the present time to account for the deposition of such enormous masses of carbonaceous matter in the earth.

If the illumination was the same as at the present time, there would hardly have been enough energy for more than threefold intensity of total CO_2-reduction, however rich the atmosphere might have been in this gas.

The general lesson to be learned from these limitations of assimilation in nature would seem to be that the biological advantage which plants gain when dwelling in the brightest habitats, is not increased assimilation, but probably increased warmth. This may be the explanation of the fact noted by Wiesner in his studies on "Lichtgenuss," that shade plants are abundant in the tropics, but gradually fail in northern latitudes, and are absent in arctic vegetation.

VIII.—*Conclusions.*

There are three conspicuous factors which control the amount of assimilation of carbon dioxide that a leaf can perform: (1) the intensity of the illumination, (2) the temperature of the leaf, and (3) the pressure of the CO_2 in the surrounding air. If the illumination is feeble, though the other factors are favourable, then the amount of photosynthesis will be kept down and light will be a "limiting factor" to the process. So similarly with the CO_2-supply, and also, as has been shown in the previous paper of this series, with the temperature. For each temperature there is a definite amount of assimilation that a leaf can perform and no more. For a given plant these amounts are very constant; at high temperatures the high rate of assimilation cannot be maintained for long, and a "time factor" comes in to complicate the relation.

The present work is an attempt to interpret the quantitative variations of carbon-dioxide assimilation, under natural or semi-natural conditions, in terms of these three chief factors.

When an increase of assimilation follows an increase in the sun's radiation incident on a leaf, it requires special investigation to determine whether the effect is actually due to the increase of light or to the increase of temperature.

Single leaves of cherry-laurel or *Helianthus* were enclosed in a glass chamber sunk in a glass water-bath, of which the temperature could be controlled, and hourly or half-hourly estimations made of their assimilation under all varieties of natural illumination. The exact temperature of the leaf was determined thermo-electrically all through the experiments, and the illumination was either the natural diurnal sequence in the open air or selected illumination, *e.g.*, diffuse light alone, full sunshine alone, or definite fractions of sunshine. The current of air through the leaf chamber was enriched with CO_2, so that inadequate supply of this gas might never be a limiting factor.

To test whether an observed assimilation-value is limited by light or by temperature, it suffices to change the intensity of each factor separately. Thus, in the case that temperature is limiting, circulation of hotter water through the bath sends up the assimilation without any increase of illumination, while, if the temperature is carefully kept constant, the illumination can be increased to any extent without causing a rise of assimilation. Assimilation in shade and in sunshine was analysed in this way.

With a leaf exposed to diffuse daylight only, the amount of assimilation is a measure of the light if the temperature is kept high, and then the assimilation will fall all through the afternoon with the waning light. If the temperature is low, then assimilation is thus limited, and remains uniform all through the varying light. Real assimilation persists until sunset. The diffuse light may have a very high photosynthetic intensity when the sky is covered with white reflecting cumuli or with a thin haze.

With a leaf kept exposed normally to the sun throughout the day a very rapid rise of assimilation sets in at sunrise, but the amount of assimilation is generally limited by the temperature, since the illuminating effect of the sun exceeds its heating effect, especially when the leaf is included in a bath of circulating water.

To apply to assimilation in free air the data obtained with a leaf in a water-bath, it is necessary to know the actual internal temperatures that leaves attain in sun and shade in the open air. Thermo-electric measurements with leaves of cherry-laurel, exposed normally to brilliant insolation, showed that the internal temperature may rise more than 10° above that registered by a bright mercury thermometer in the sun: a leaf exposed in a closed glass vessel may be heated a further 10°.

At natural temperatures neither *Helianthus* nor cherry-laurel is capable of utilising for assimilation the whole of the appropriate radiation in full sunlight, so experiments were made to determine what fraction of this is used at a given temperature. For cherry-laurel it was found that 0·28 sun, near noon, near the middle of August, can reduce 0·0116 gramme CO_2 per hour per 50 sq. cm. of leaf area. With *Helianthus*, 0·62 sunlight, at about the same time, reduced 0·0224 gramme. These indicate for full sunlight the photosynthetic values of 0·0414 and 0·0361 on the two occasions. Making a correction for the time factor, the values come out higher and still closer together.

The highest assimilation actually measured (Experiment XVI) was 0·0290 gramme CO_2 per 50 sq. cm. leaf area per hour for *Helianthus* at 29°·5 C. This is about 2900 c.c. CO_2 per square metre per hour.

The nature of the specific assimilational characteristics of different types of leaf was investigated, and it is shown that:—

1. Equal intensities of light, incident upon equal areas of different leaves, produce, when light is the limiting factor, equal *amounts* of assimilation. This is proved to be true within 5 per cent. for such diverse leaves as cherry-laurel, *Helianthus, Tropæolum, Bomarea, Aponogeton.*

2. All leaves have the same economic coefficient of photosynthesis, using the term in the narrow sense to refer only to those radiations specific to this process.

3. At low temperatures different leaves, such as *Helianthus* and cherry-laurel, have similar assimilation-maxima, but at high temperatures the maxima diverge. At 29°·5 C. *Helianthus* can assimilate twice as much CO_2 as cherry-laurel.

4. This is harmonised with the first law by showing that *Helianthus* requires just twice as much light to attain this double assimilation.

5. The essential difference between these two leaves lies in their having different coefficients of acceleration of their assimilation-activity with increase of temperature.

6. From this it results that the two leaves utilise different fractions of sunlight at any temperature: this fraction is to be found for any temperature by dividing the assimilation-maximum of the particular leaf at that temperature by the photosynthetic value of the sunlight.

7. There is no optimum intensity of light for assimilation.

In Nature, the high values of assimilation that are obtained experimentally cannot take place, because assimilation is limited by the small pressure of CO_2 in the atmosphere.

There is thus a general waste or diversion into other channels of the photosynthetic energy in sunlight and diffuse light.

A table is constructed, showing the amount of energy unutilised at various temperatures by different leaves, with such noontide illumination in sun and in shade as actually prevailed on a definite day.

Were the CO_2 in the atmosphere augmented moderately, then this would cease to be the limiting factor in the shade generally, and also in feeble sunlight. Temperature, unless unnaturally high, would then limit assimilation in Nature, and still prevent bright insolation producing its full effect.

[*Notes added July* 25, 1905. While this paper has been in the press, there has been published in this Journal an extensive paper on a cognate subject by Dr. Horace Brown and Mr. Escombe, entitled "The Physiological Processes of Green Leaves, with Special Reference to the Interchange of Energy between the Leaf and its surroundings" ('Roy. Soc. Proc.,' vol. B 76, 1905, pp. 29 to 111). The following notes are now added to indicate as briefly as possible the points of contact between that paper and our own.

As a point of general significance it must be noted that the expression "*amount* of assimilation" has not the same meaning in the two papers. We use the unqualified words to mean the total photosynthetic work done in any time, part of the CO_2 for this being, of course, drawn from the external air and part from the concurrent respiratory activity of the leaf. Brown and Escombe intentionally leave respiration entirely out of account, and the unqualified word "assimilation" with them refers only to the intake of CO_2 from without.

A (see p. 412). Brown and Escombe have published detailed balance sheets (pp. 100 to 111) of the "Thermal Interchange" between leaves and their surroundings under various conditions of natural illumination. One interesting feature of these tables is the estimation of the internal temperature of the leaf. The degree of accuracy of this estimation is dependent upon the degree of accuracy of the six following determinations: (1) the coefficient of absorption of radiation by the leaf; (2) the specific heat of the leaf; (3) the energy being expended in transpiration; (4) the energy being expended in photosynthesis; (5) the thermal emissivity of the leaf; and (6) the effect upon this function of the particular velocity of movement of the surrounding air at the time. The temperatures arrived at by these truly admirable calculations are, however, in no case checked by actual determinations. The temperatures given in these tables for leaves in the sun in the open air are never more than 2° C. above the shade temperature of the air, while our few direct measurements with cherry-laurel leaves, brilliantly insolated, indicated 7° to 16° C. above the thermometer in the shade. Our method is not unexceptionable, but it does not seem probable that this divergence is to be wholly accounted for by the combined effects of faults of method, exceptional insolation, and thickness of leaf used. (Brown and Escombe employed only types of leaf which were thin.)

B (see p. 435). Our *a priori* criticism of the method of fractioning light by the use of "rotating sectors" has not the cogency given to it in the text. Consideration of a number of experiments, in which this method was employed by Brown and Escombe, allows one to see that the leaf behaves in assimilation as the eye does in vision, and that halving the light *in time* has, with all strengths of illumination, the same effect as halving it in intensity. The interest of the matter therefore shifts to the determination of the induction period and to finding at what slowness of rotation of the sectors the leaf begins to distinguish between the two methods of reduction of illumination.

C (see p. 454). Brown and Escombe have made special inquiry into the trustworthiness of Sachs' "increase of dry weight method" of estimating assimilation, and their verdict is very unfavourable.

The value of 0·0077 gramme that we have adopted for assimilation in the open air when the CO_2-pressure is the limiting factor, was obtained by Brown and Morris by this method, and is therefore subject to suspicion and is probably too high.

The new data for arriving at this most important value, brought forward by Brown and Escombe, and based on measuring the intake of CO_2 by a leaf in a glass chamber in a rapid current of ordinary air, show a very wide range of variation even for the leaves of a single species (0·0016 to 0·0047 gramme per 50 sq. cm. for *Polygonum Weyrichii*). Primarily, the degree to which the stomata are open and, secondarily, the magnitude of the concurrent respiratory production of CO_2, would seem to be the important factors in disturbing the inflow of CO_2 from without.

The highest value thus arrived at is 0·0047 for a detached leaf, and as attached leaves with stomata less widely open give smaller values, the number adopted by us may be much too high. This would increase the waste of photosynthetic radiation in Nature, but the information at present available is not sufficient to allow us to readjust the table on p. 455].

Note on the Mechanics of the Ascent of Sap in Trees.

By Professor J. LARMOR, Sec. R.S.

(Received June 29, 1905.)

The following remarks, relating to one of the most powerful and universal of the mechanical operations of organic nature, are based mainly on the numerous experimental results reported in Dr. A. J. Ewart's recent memoir.* Their chief object is to assert the view that we are not compelled to suppose the sap, in the column of vessels through which it rises, to be subject to the great actual pressure, amounting in high trees to many atmospheres, that is sometimes postulated. It is hardly necessary to remark that the problem of the rise of sap is one of mechanics, in so far as concerns the mode of the flow and the propelling power.

Contrary to the view above referred to, it seems not unreasonable to consider that the weight of the sap in each vessel is sustained in the main by the walls and base of that vessel, instead of being transmitted through its osmotically porous base to the vessels beneath it, and thus accumulated as hydrostatic pressure.

We could in fact imagine, diagrammatically (as happens in ordinary osmotic arrangements) a vertical column of vessels, each provided, say, with a short vertical side-tube communicating with the open air, in which the pressure is adjusted from moment to moment, and yet such that the sap slowly travels by transpiration from each vessel to the one next above, through the porous partitions between them; provided there is an upward osmotic gradient, *i.e.*, if the dissolved substances are maintained in greater concentration in the higher vessels.* This difference of density must be great enough, between adjacent vessels, to introduce osmotic pressure in excess of that required to balance the head of fluid in the length of the upper one, into which the water has to force its way. Thus, in comparing vessels at different levels, the sap must be more concentrated in the upper ones by amounts corresponding to osmotic pressure more than counteracting the total head due to difference of levels, in order that it may be able to rise. As osmotic pressure is comparable with gaseous pressure for the same density of the molecules of the dissolved substance, the concentration required on this view is considerable, though not very great.

---

* 'Roy. Soc. Proc.,' vol. 74, p. 554; 'Phil. Trans.,' B, vol. 198, p. 41.

Such a steady gradient of concentration could apparently, on the whole, become self-adjusting, through assistance from the vital stimuli of the plant; for concentration in the upper vessels is promoted by evaporation. Yet pressures in excess or defect of the normal atmospheric amount might at times accumulate locally, the latter giving rise to the bubbles observed in the vessels, through release of dissolved gases.

It may be that this assumes too much concentration of dissolved material in the sap, *as it exists inside the vessels of the stem,* to agree with fact. In that case the capillary suction exerted from the nearest leaf surface might be brought into requisition, after the manner of Dixon and Joly, to assist in drawing off the excess of water from the vessels. The aim proposed in this note is not to explain how things happen, which is a matter for observation and experiment, but merely to support the position that nothing abnormal from the passive mechanical point of view need be involved in this or other vital phenomena.

---

* Thus in an ordinary osmotic experiment with a U-tube, the percolation of water through the plug gradually *produces* a difference of hydrostatic pressure on its two faces, which is *sustained* by the fixity of the plug itself, but would be at once neutralised if the plug were free to slide in the tube. This increase of volume of the salt-solution, by the percolation of pure water into it, is on the van't Hoff analogy correlated with the free expansion of the molecules constituting a gas. It goes on with diminished speed under opposing pressure, until a definite neutralising pressure is reached, inaptly called the osmotic pressure of the molecules of the solute, which just stops it, while higher pressures would reverse it. The stoppage is due to the establishment of a balance between the amounts of water percolating one way under osmotic attraction, and the opposite way under hydrostatic pressure. The pressure established, *e.g.*, in an organic cell immersed in salt-solution, is thus really the reaction which is set up against the osmotic process. That process itself is perhaps more directly and intelligibly described as the play of osmotic affinity or attraction, even though it must be counted as of the same nature as the affinity of a gas for a vacuum. *Cf.* 'Proc. Camb. Phil. Soc.,' January, 1897, or Whetham's "Theory of Solution," p. 109.

As regards estimating the amount of flow, at first sight it may not appear obvious, *a priori*, that the transpiration through a porous partition or membrane, due to osmotic gradient, is equal or even comparable in amount to what would be produced, with pure water, by a hydrostatic pressure-head equal to the difference of the osmotic pressures on the two faces of the partition. But more exact consideration shows that on the contrary osmotic pressure is *defined* by this very equality;* it is that pressure-difference which would produce such an opposite percolation of water as would just balance the direct percolation due to the osmotic attraction of the salt solution.

It would, however, appear that the great resistance to flow offered by what botanists call Jamin-tubes, viz., thin liquid columns containing and carrying along numerous broad air-bubbles, is conditioned mainly by the viscosity of the fluid, and involves only indirectly the surface-tension of the bubbles. In fact the resistance to flow may be expected to remain much the same if each bubble were replaced by a flat solid disc, nearly but not quite fitting the tube. Its high value arises from the circumstance that the mass of liquid between two discs moves on nearly as a solid block when the flow is steady, so that the viscous sliding has to take place in a thin layer close to the wall of the tube, and is on that account the more intense, and the friction against the tube the greater. The increased curvature of the upper capillary meniscus of the bubble is thus merely a gauge of the greater intensity of the viscous resistance instead of its cause, and modification of the surface-tension cannot be involved as a propelling power. The experimental numbers given by Dr. Ewart show that, even where the vessels are largely occupied by bubbles, the greater part of the resistance to active transpiration still resides in the partitions between them.

If the osmotic gradient, assisted possibly by capillary pull at the leaf-orifices, is insufficient to direct a current of transpiration upward, *capillary* alterations inside the vessels, arising from vitally controlled emission and absorption of material from the walls, cannot be invoked to assist: rather it must be *osmotic* alterations from one vessel to the next, of, so to speak, a peristaltic character, that might thus come into play. But any such alteration (of either kind) will involve local supply of energy. Is there a sufficient fund of energy, latent in the stem, to provide permanently the motive power for the elevation of the sap? In what form could this energy get transported there? The energies of the plant-economy come from the sunlight absorbed by the leaves. The natural view would appear to be that the work required to lift the sap is exerted at the place where the energy is received, and that it operates through extrusion of water by evaporative processes working against the osmotic attraction of the dissolved salts; while the maintenance of equilibrium along the vessels of the balanced osmotic column, with its semi-permeable partitions, demands that an equal amount of water must rise spontaneously to take the place of what is thus removed.

---

* See preceding footnote.

The subject might, perhaps, be further elucidated by observation of the manner in which the flow is first established at the beginning of the season, or possibly by experiments on the rate at which water would be absorbed by a wounded stem high above the ground.

against the osmotic attraction of the dissolved salts; while the maintenance of equilibrium along the vessels of the balanced osmotic column, with its semi-permeable partitions, demands that an equal amount of water must rise spontaneously to take the place of what is thus removed.

The subject might, perhaps, be further elucidated by observation of the manner in which the flow is first established at the beginning of the season, or possibly by experiments on the rate at which water would be absorbed by a wounded stem high above the ground.

On the Cytology of Apogamy and Apospory.—II. Preliminary Note on Apospory.

By Miss L. Digby.

(Communicated by Professor J. B. Farmer, F.R.S. Received April 15,—Read May 11, 1905.)

Apospory is the direct vegetative process which leads from the sporophyte to the gametophyte without the intervention of spores. This phenomenon has for a long time been known to occur in mosses* and ferns,† and several writers have described its morphological characters, but its cytological history has hitherto remained unrecorded. In a previous note‡ the cellular features of apogamy were briefly described, and it is here proposed to treat the problem of apospory in a similar manner. The following remarks are limited to the study of apospory in *Nephrodium pseudo-mas* Rich. var. *cristata apospora,* Druery,§ but it is of interest to note that within the limits of a (probably) single, but highly variable species, almost all grades of apospory and apogamy, with the exception of true parthenogenesis, have been encountered. Our thanks are especially due to Mr. C. T. Druery, who has kindly supplied us with the original fronds, and to the Curator of the Chelsea Physic Garden, who has most carefully cultivated the plants with excellent results.

---

* N. Pringsheim, "Vegetative Sprossung der Moosfrüchte," 'Monatsb. Akad. Wiss., Berlin,' July 10, 1876, pp. 425 to 429.

E. Stahl, "Ueber künstlich hervorgerufene Protonema-bildung an dem Sporogonium der Laubmoose," 'Bot. Zeitg.,' vol. 34, 1876, pp. 689 to 695.

N. Pringsheim, "Ueber Sprossung der Moosfrüchte und den Generationwechsel der Thallophyten," 'Jahrb. für wiss. Bot.,' vol. 11, 1878, pp. 1 to 46.

† C. T. Druery, "Observations on a Singular Mode of Development in the Lady Fern (*Athyrium Filix-fœmina*)," 'Jour. Linn. Soc. Bot.,' vol. 21, 1884, pp. 354 to 358 and pp. 358 to 360.

F. O. Bower, "Apospory and Allied Phenomena," 'Trans. Linn. Soc. Bot.,' 2nd series, vol. 2, Part 14, July, 1887, pp. 301 to 326.

‡ J. B. Farmer, J. E. S. Moore, and L. Digby, "Preliminary Note on Apogamy," 'Roy. Soc. Proc.,' vol. 71, 1903, pp. 453 to 457.

§ See "*Lastrea pseudo-mas* var. *cristata apospora,*" C. T. Druery, 'Book of British Ferns,' p. 99.

The aposporal character of *Nephrodium pseudo-mas* var. *cristata apospora* appears to have been first noted by Dr. F. W. Stansfield. It occurred as a sport in a very damp fernery. Our fronds were pegged down in pans of moist earth, and the cultures have been grown in a greenhouse kept at an average temperature of 55° to 60° Fahr. in the winter and of 65° to 70° Fahr. in the summer. It was found that no aposporous growths appear until the fronds have been pegged down, and in each case the fronds were severed from the plant, as this was found to greatly encourage the production of the growths. The aposporous growth is rapid and prolific; fronds treated during the spring and summer showed prothalli, bearing embryos, in three weeks' time. By the autumn these young plants were sufficiently matured to have their leaves layered, and these shortly exhibited the same characteristic feature. It is immaterial as to which surface of the frond is in contact with the soil. So far as has been ascertained this fern remains very constant in character, and continues to breed true. In the plants grown at Chelsea, no trace of a sporangium or sorus has appeared on any of the leaves.

The prothallial growth originates either from the surface, or more frequently from the edge (fig. 1) of the frond. It is at first discernible as a small out-

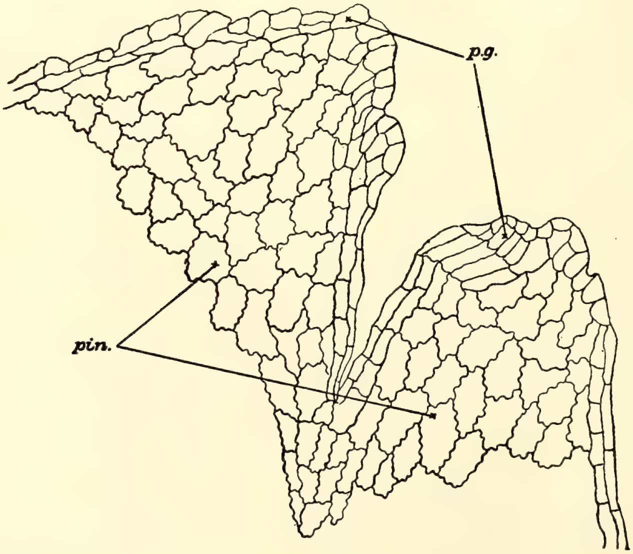


Fig. 1.—Prothallial growth from apices of pinnule. *p.g.*, prothallial growth. *pin.*, pinnule.

growth caused by the division both of the marginal cells of the leaf and of those cells lying immediately within the margin. As the growth proceeds, it is clearly distinguished as a more or less continuous sheet of delicate tissue formed of somewhat rectangular cells. In due course, owing to rapid growth at certain points, the typical prothalloid shape is assumed. The study of many pinnules shows the apex either produced into a single prothallus (fig. 2) or crowned by a cluster of prothalli, or else the edge is beset with isolated groups. The prothalli on the surface of the leaf may be closely approximated to, or independent of, the vascular tissue. The majority of the prothalli are typically regular in shape, but irregular ones are by no means uncommon. The regular prothalli are normal in appearance, except that they have no well-developed cushion. Antheridia are frequently found, even when the prothalli are comparatively young, but archegonia have never been seen. The prothalli generally show the presence of an embryo in various stages of growth. It is situated in the position normally occupied by the cushion. The irregular prothalli already alluded to vary greatly in shape, and apparently seldom bear antheridia, and never give rise to an embryo.

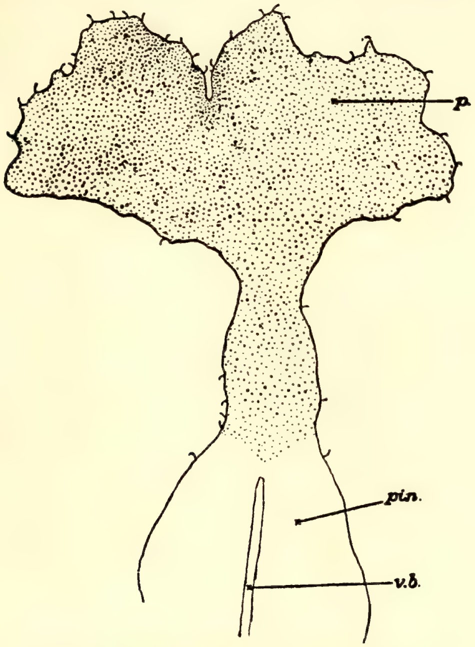


Fig. 2.—Prothallus grown from tip of pinnule. *p.*, prothallus, *pin.*, pinnule, *v.b.*, vascular bundle.

The principal cytological interest in the prothalli centres in the number of the chromosomes, and in a comparison between the prothallus and the sporophyte in this respect. The mean of a considerable number of actual countings in the gametophyte is 43 (fig. 3, *a*, *b*, *c*). This calculation is certainly too low owing to the difficulty of realising every individual when dealing with high numbers; 50 is probably nearer the actual figure.

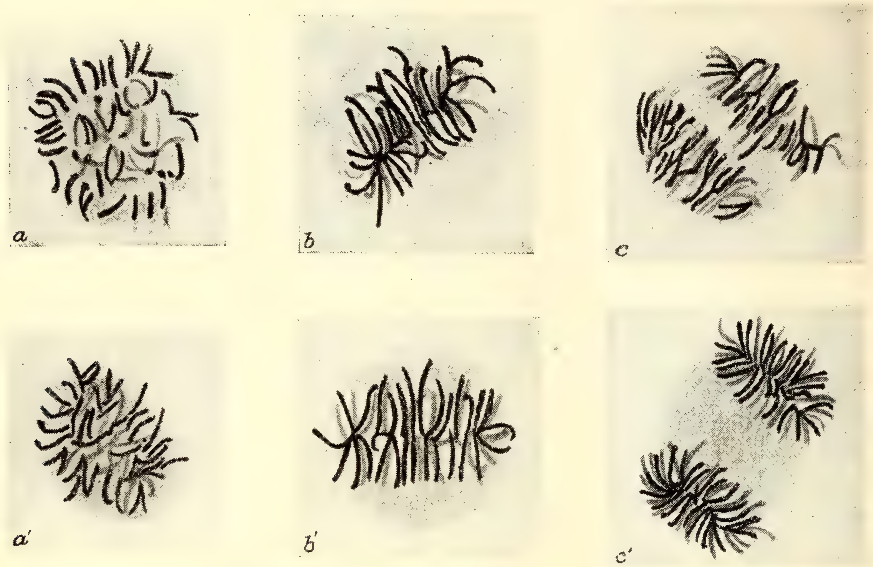


Fig. 3.—Diagrams of nuclear divisions. *a b c* in prothallus. *a′ b′ c′* in embryo.

The embryo arises as a direct vegetative outgrowth from the prothallus, and, when very young, consists of a rounded mass of cells in which the apical cells of the cotyledon, stem and root, are clearly recognisable. The longitudinal section of an older plant is of a normal type, except for the absence of a foot. The nuclear divisions have been carefully worked out, and, as in the prothallus, the average number of chromosomes present at each mitosis has been taken from several countings. The mean of the numbers obtained is 41, but as in the case of the prothallial nuclei, this is certainly too low (fig. 3, *a′*, *b′*, *c′*). The absolute number is not, however, of great importance, but the close approximation in the results of the chromosome countings of the prothallial and sporophytic nuclear divisions, undoubtedly proves that there is *no reduction* during the transition of the sporophyte to the gametophyte generation. A similar result has been obtained in *Athyrium Filix-fœmina* var. *clarissima* Jones, where the aposporous growth is formed in relation to young but abortive sporangia.* Here, however, the absence of reduction is accompanied by cytological features, indicating a transient condition characteristic of an earlier phase of the heterotypical mitosis, which may throw some light on the anomaly. A discussion of these results will appear in the final memoir. It is interesting to note that Professor Strasburger has placed a similar interpretation on the phenomenon of apogamy in *Eualchemilla*,* which was first described by Murbeck. Here, too, there is no reduction either in the formation of the embryo sac or of the ovum, the latter giving rise, without fertilisation, to the apogamously formed embryo.

---

* Bower, *loc. cit.*

The prothalli of the two apogamous varieties, *Nephrodium pseudo-mas* Rich. var. *polydactyla* Wills, and *Nephrodium pseudo-mas* Rich. var. *cristata apospora* Druery, exhibit two striking differences. Whereas in the former nearly all the prothalli, except very young ones, have a strand of vascular tissue extending throughout the greater part of their length, in the latter only two cases of feebly-developed tracheides have been seen. Again, in *Nephrodium pseudo-mas* var. *polydactyla*, migrating nuclei, some of which have been seen to fuse, are a characteristic feature.† Out of a large number of prothalli it was found that about 73 per cent. of the young ones exhibit phases of nuclei passing from one cell to another. As this fern produces fertile spores, it is almost certain (it is hoped shortly to settle this point) that there is a true reduction during the division of the spore mother cells, the doubled number characterising the sporophyte is apparently brought about by the migration and fusion of prothallial nuclei. In *Nephrodium pseudo-mas* var. *cristata apospora*, out of 80 prothalli examined, only two showed possible cases of nuclear migration, and these were open to doubt as regards their interpretation.

The reason for the absence of fusion in other cases is obvious, for the prothalli of *Nephrodium pseudo-mas* var. *cristata apospora*, as we have seen, already possess the full complement of somatic chromosomes. Hence there is no need for the fusion of two nuclei which, by their union, double the number of chromosomes.

I am deeply indebted to Professor J. Bretland Farmer, and to Mr. J. E. S. Moore for their constant help and advice. Professor Farmer has most kindly allowed me to use his material, and has superintended the work throughout.

---

* E. Strasburger, "Die Apogamie der *Eualchimillen*," 'Jahrb. für wiss. Bot.,' Leipzig, vol. 41, 1904, pp. 88 to 164, Pl. I to IV.

† Farmer, Moore, and Digby, *loc. cit.*

The Pharmacology of Indaconitine and Bikhaconitine.

By J. Theodore Cash, M.D., F.R.S., and Wyndham R. Dunstan, M.A., LL.D., F.R.S.

(Received May 24,—Read June 8, 1905.)

The present paper deals with the physiological action of two new "aconitines," which have been isolated at the Imperial Institute from two varieties of Indian aconite. One is an alkaloid, which has been named indaconitine. It was found in the roots of the Indian aconite, called by Bruhl, *Aconitum napellus*, var. *hians*, since identified by Stapf as a new species, which has received the name of *Aconitum chasmanthum. Aconitum chasmanthum* being abundant in India, the highly toxic alkaloid derived from this plant has been called "indaconitine," a name appropriate to the properties of this alkaloid, which closely resemble those of aconitine derived from the common European aconite, *Aconitum napellus.*

The other alkaloid has been named "bikhaconitine," being derived from one of the highly poisonous forms of aconite known in India under the vernacular name of "Bikh." This aconite was named by Bruhl, *Aconitum ferox*, var. *spicatum*, but has been re-named *Aconitum spicatum* by Stapf, who regards it as a distinct species.

The chemistry of these two alkaloids, which has been fully worked out at the Imperial Institute, will be described by one of us in a separate communication. It will be sufficient to allude here to the leading chemical characters of the two substances.

Indaconitine differs only slightly from aconitine in its composition and properties, but in several respects these differences are well marked. Indaconitine crystallises well, but its usual crystalline habit is distinct from that of aconitine, although the crystallographic characters of the two substances are very similar, and they may prove to be isomorphous. Most of the salts crystallise readily, and the hydrobromide has been employed for the study of the physiological action of the alkaloid.

Like aconitine, indaconitine undergoes hydrolysis in two stages. Partial hydrolysis leads to the separation of a molecule of acetic acid, and the formation of a base which has been named benzoyl-pseudaconine. This substance on further hydrolysis furnishes one molecule of benzoic acid, and a base which proves to be identical with pseudaconine, the ultimate hydrolytic product of pseudaconitine derived from forms of *Aconitum ferox*, the chemistry and pharmacology of which have been described in previous papers. Indaconitine therefore contains the acetyl and benzoyl groups present in aconitine of European origin, associated with the basic nucleus of the Indian pseudaconitine. Its pharmacology as described in the present paper corresponds with its chemical relation to these two alkaloids.

Bikhaconitine closely resembles pseudaconitine, but is chemically distinct from it. The alkaloid and its salts crystallise well. Similarly its derivatives somewhat resemble those of pseudaconitine, but are distinct substances. On partial hydrolysis bikhaconitine furnishes one molecule of acetic acid and veratryl-bikhaconine, which on further hydrolysis furnishes one molecule of veratric acid and bikhaconine. Bikhaconitine is therefore, chemically, the analogue of pseudaconitine, and is also its pharmacological congener. It is only slightly inferior in toxic power to pseudaconitine, which is the most poisonous aconitine yet examined.

The examination of the physiological action of indaconitine and bikhaconitine has been carried out on parallel lines with that of the alkaloids aconitine, pseudaconitine, and japaconitine, which have been previously discussed.* In each case the hydrobromide was the salt employed. It is proposed here to give very briefly, often in form of synopsis, the main results arrived at, including the dosage which is associated with a lethal action.

Indaconitine will be first considered.

INDACONITINE.

Effect upon Blood-Pressure, Pulse, and Respiration of Anæsthetised Animals (Cats and Rabbits).

There is a striking similarity with the effects produced by parallel doses of aconitine (from *A. napellus*). The phases of slowing of the pulse (with or without a slight anterior acceleration) marked quickening, and subsequent arhythmia, due to incoordinate action of auricles and ventricles, are all present, whilst similar changes in the blood-pressure, culminating in the rapid and extensive fluctuations so characteristic of aconitine, are occasioned also by indaconitine. Under ether there is little (1/6 to 1/5) or none of the primary acceleration of respiration which usually occurs in non-etherised animals (see later), but a gradual slowing, and eventually a failure of effective respiratory movements is witnessed, this condition speedily leading to a fatal issue if vigorous artificial respiration is not employed.

Vagus section and stimulation have a similar result during the toxic conditions occasioned by aconitine and indaconitine respectively, the acceleration of cardiac action, and the more effective systole due to a closer sequence of ventricular upon auricular action, with a resulting rise in the pressure being observed on stimulating during the stage of cardiac irregularity. The proportion of indaconitine capable of producing such results was 0·0011 gramme per kilogramme and upwards, administered hypodermically in a single dose. When one-half of the lethal proportion (per kilogramme) was repeated at intervals of 45′, the third dose was followed in rabbits by great irregularity of the pulse, wide fluctuations in blood-pressure, and rapid decline in respiratory frequency. The long inspiratory pause, with the forced effort at its commencement, became more marked as the toxic action progressed. This proportionate dose repeated twice at intervals of 45′ may prove lethal, though this is an exceptional occurrence.

---

* See 'Phil. Trans.,' B, vol. 190, 1898, and abstract in 'Proceedings,' vol. 62; 'Phil. Trans.,' B, vol. 195, 1903, and abstract in 'Proceedings,' vol. 68.

No estimation was made of the lethal dose of indaconitine for non-anæsthetised cats, but taking the proportion as 0·00012 which is applicable to rabbits, it was found that half this dose, administered hypodermically and repeated every 45′, was followed by death in etherised cats about 70′ after the third injection. The effects were thus developed:—

After First Injection.—Acceleration (transitory) and slowing of the pulse. Moderate fall of arterial pressure. Slight acceleration followed by slowing of the respiration.

After Second Injection.—Continued slowing of the pulse. Thereafter acceleration. Blood-pressure fairly steady. Further slowing of the respiration. Usual results of peripheral vagus and splanchnic stimulation.

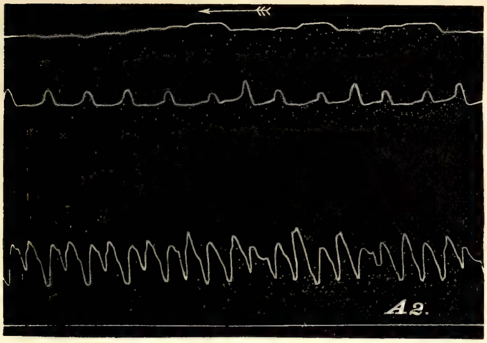
After Third Injection.—Irregularity of the pulse increasing as time progressed. The heart greatly accelerated, attaining 200 per minute. Immediately before death, and when the blood-pressure amounted to 28 mm. of mercury, the rhythm became regular. In the earlier part of this period, vagus stimulation co-ordinated the action of the auricles and ventricles, temporarily raising the blood pressure, but this effect was lost later. Splanchnic reaction was never entirely abolished. The respiration, at first very slow but effective, became weaker and was suspended. Artificial respiration served to prolong life for over 20′. The tracings (A_{1-3}) are taken from an experiment in which registration of carotid pressure and contraction of the left auricle and ventricle were taken simultaneously.

A_1. Before injection.

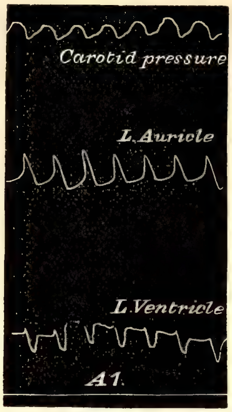
A_2. 50′ after the third injuction of 0·5 lethal dose of indaconitine. (The injections occur every 45′.)

A_3. 70′ after the third injection, and 5′ before death.

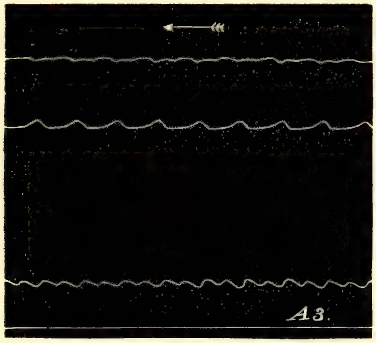
50 mins. after third injection of one-half of lethal dose of indaconitine.

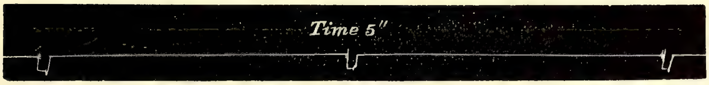


Before injection.



70 mins. after. 5 mins. before ex. leth.



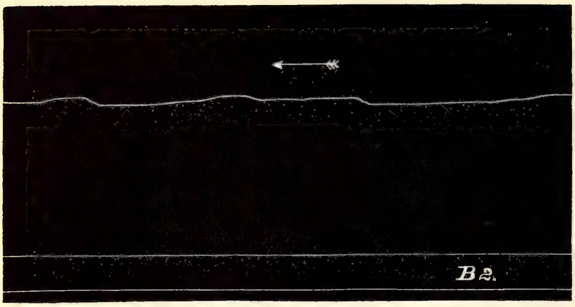


Antagonism of Atropine.

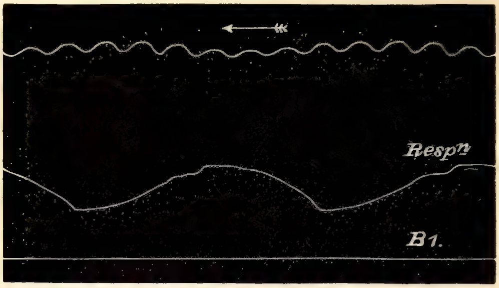
The antagonism of atropine and indaconitine is of the same character as that which has been described as existing between atropine and aconitine. Not only is the irregularity of the pulse reduced (the heart being slowed and the sequence of ventricular action restored), but the blood-pressure is increased and steadied, whilst the respiration, which may have been abolished as an effective function, is rapidly reinstated, the original speed being attained or approached.

In the following experiment (B_1), injections were made by the femoral vein of an ætherised cat of repeated small doses of indaconitine. In 50′ a proportion of 0·00016 gramme per kilogramme had been totalled; irregularity of the heart was present, and respiration was represented only by

After 0·00016 per kilogramme indaconitine.

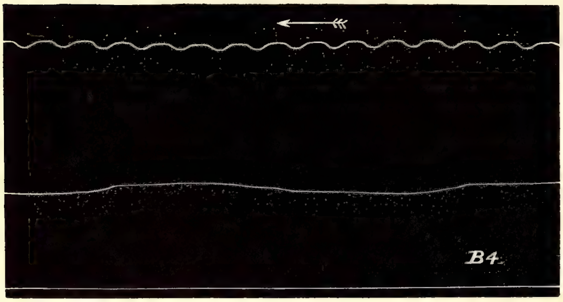


Before injection.

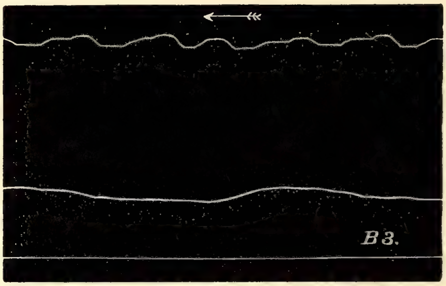


an occasional and inefficient movement, rendering artificial insufflation necessary. In the following 25′ an additional injection of 0·000022 indaconitine was made (B_2). Five minutes later the heart was beating very rapidly (over 300) and irregularly (36 impulses per minute in the carotid), whilst the blood-pressure was falling so fast that a lethal issue was obviously impending. Injection of 0·006 gramme of atropine sulphate was now made by the femoral vein, and the serious symptoms soon began to abate, so that 20′ later carotid pressure was 56 mm., carotid impulses (though very irregular) 77, and respirations 15 per minute. During the succeeding 25′ further injections of atropine sulphate amounting to 0·0089 gramme were made; the pulse remained irregular, and often bigeminal in character; pressure 65 mm., and respirations 50 per minute (B_3). A further injection of 0·004 gramme of atropine restored the regular beat of the pulse to 154 per minute, the pressure rising

Complete antagonism by atropine.



Partial antagonism by atropine.



Time, 5 secs.

to 96 (B_4). Thus the effects of indaconitine, amounting to 0·000182 gramme of the alkaloidal salt per kilogramme, were fully antagonised by 0·018 gramme of atropine sulphate (0·01 per kilogramme) in the course of 65′. The pulse and pressure having remained steady for a period of 40′, the interrupted injections of indaconitine were resumed, when it required no less than 0·00027 gramme per kilogramme (administered in the course of 50′) to reproduce a distinct aconitine effect. It is probable, therefore, that atropine had been used in excess of the antidotal equivalent required by the original indaconitine injection.

Action of Indaconitine on Rabbits.

A single hypodermic injection of 0·00008 gramme per kilogramme of indaconitine produced acceleration of the respiration, then slowing of respiratory rhythm to one-half or thereabouts of the original rate, salivation, dilatation of the pupil and a fall of rectal temperature amounting to about 1·5° C. These effects were transitory, as salivation lasted for less than 30′, whilst respiration had returned to, or nearly, to the original, and the temperature was beginning to recover 90′ after injection had been made.

0·00011.—Sharp acceleration of respiration succeeded by slowing to 14 or 16 per minute. Salivation ushered in by active chewing and retching movements. Early pupillary dilatation. Marked dyspnœa, with some retraction of thorax on inspiration; occasional dyspnœal spasm. Moist large crepitations in air passages. Much paresis of limbs. The total fall of temperature amounted to between 2 and 2·5° C. The symptoms were abating in 110′.

0·00012 per kilogramme.—Transitory respiratory acceleration followed by great slowing. Salivation and dilatation of the pupil in 15′. Considerable paresis and respiratory spasm. Death occurs in 50′ to 60′.

Lethal Dose.—Although an occasional lethal result may follow doses of the indaconitine salt proportional to 0·0001 per kilogramme, it is not until one-fifth more is given that the result becomes almost invariable. The lethal relationship stands accurately at 0·00012 gramme per kilogramme—only once has this dose been exceeded, with a different issue.

Repeated Administration of Indaconitine.—On Temperature and Respiration.

Indaconitine causes an acceleration of respiration immediately after injection of doses of one-third of the lethal proportion and upwards, especially if the original respiratory rhythm had been less than 60 per minute. But if the original rate is twice as rapid, then there may be an absence of acceleration. The tendency to acceleration becomes less as the proportion of aconitine becomes smaller.

In all cases, whether there be a primary rise or not (after one-third of the lethal and upwards), a fall in the respiratory rate follows injections of indaconitine. This may amount to one-half of the original, after a dose of one-half of the lethal, or a reduction to from one-quarter to one-fifth after five-sixths of the lethal. In the latter case the movements are distinctly dyspnœal, retraction of the chest wall, fixation of the thorax and relative prolongation of the inspiration being present. The acceleration which sooner or later succeeds to this slowing (the dose being sub-lethal) is often to a rapidity of rhythm much beyond the original. This is more particularly the case when doses of about half lethal are given than when the proportion is larger. The acceleration occurs earlier in the course of readministrations of indaconitine than after equivalent doses of bikhaconitine.

Repeated Administration of Indaconitine at Stated Intervals to Rabbits.

Effect on Temperature and Respiration.

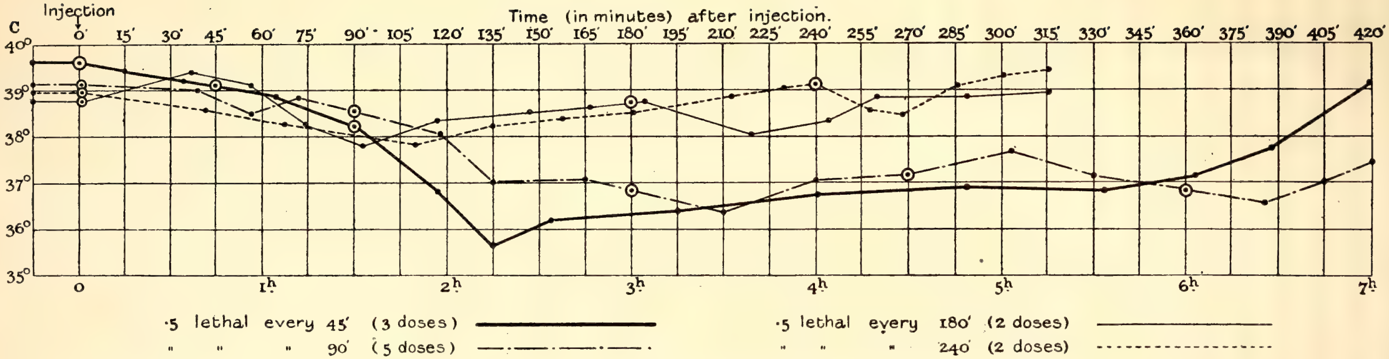
Fraction of lethal proportion per kilo. body weight.	When repeated.	How often.	Total proportion to the lethal.	Greatest fall of temperature (C.).	Subsequent course of temperature.	Note on respiration.
$\frac{1}{6}$	mins. 45	7	$1\frac{1}{6}$	1°·0	Greatest reduction after second injection. Thereafter broken rise.	
„	60	6	1 ·0	0 ·9	Greatest after second injection.	
$\frac{1}{4}$	45	8	2 ·0	2 ·3	Greatest after fifth injection, when broken rise ensues.	Eventual acceleration of respiration, more rapid than in case of bikhaconitine.
„	60	6	1 ·5	1 ·2		
$\frac{1}{2}$* (Diag. A)	45	3	1 ·5	3 ·8	Greatest after third injection. In 420′ is still −1° C.	Respiration reduced to 13 per minute. Rises with the temperature.
„ (Diag. A)	90	3	1 ·5	2 ·8	Greatest after second injection.	
„	120	3	1 ·5	1 ·6	Greatest after first injection.	
„ (Diag. A)	180	2	1 ·0	1 ·0	Rise of 0·7° C. Fall greatest after first injection.	
„ (Diag. A)	240	2	1 ·0	1 ·2	Greatest after first injection.	

* Such administrations (0 ·5 lethal twice repeated at intervals of 45′) were made in three experiments, in one of these with lethal result (see p. 470).

Toxic Action of Indaconitine in Progressive Doses towards Frogs.

0·0006 gramme per kilogramme weight of *R. temporaria.* Some excitement (soon subsiding) after injection. No marked impairment of reflexes. Acceleration of respiration succeeded by slowing.

DIAGRAM A.



Effect of Indaconitine on the Internal Temperature of Rabbits. Administrations are indicated by the mark ⊙.

0·0009.—Considerable excitement with irregularity of reflexes in limbs and inability to assume ventral position when placed on dorsum. The lungs are in the main widely inflated; respiratory movements occasional.

0·0012.—After initial excitement with frothing there is slowing and suspension of respiration—gaping. Reflexes much impaired and very uncertain. Prolonged inability to get off dorsum. Recovery not complete for five days.

0·0013.—This is a hyperlethal proportion. After excitement there is rapid failure of voluntary movement. Reflexes are diminished and uncertain in half an hour after injection. Respiration, after brief acceleration, is slowed and abolished. Heart's action becomes incoordinate. If death occurs (from cardiac failure) in five to six hours, some reflex may occasionally be elicited even when circulation has ceased, but if the lethal issue is postponed till some hours later, the limb reflexes disappear first, the body being limp and flaccid. The ventricle shows characteristic sacculations containing blood. The gastrocnemius is excitable to direct stimulation, usually the response to indirect stimulation is impaired.

The *lethal dose* of indaconitine varies in different seasons of the year. For *R. temporaria* in June and July a proportion of 0·00120 gramme per kilogramme was lethal on several occasions, and the proportion of 0·00125, which may be regarded as the lethal, was only twice exceeded (0·0014 and 0·00145) with subsequent recovery.

Action of Indaconitine upon the Cardiac Rhythm.—Lethal and slightly hyperlethal doses of indaconitine cause acceleration of the heart of brainless frogs, amounting to from four to eight beats per minute. The acceleration may be progressive after injection, or may be preceded by slight slowing (two to three per minute). After a duration, which varies largely according to the dose, acceleration passes into irregular rhythm with incoordinate action, the ventricle being chiefly affected. It is after this condition has lasted for a time that the characteristic "pouching" of the ventricle is produced by indaconitine, as it is by all the aconitines hitherto examined in this research. Subsequently, after lethal doses, a progressive slowing and enfeeblement of the ventricular beat occurs, until spontaneous action ceases. At this time the auricles are beating with some degree of regularity at a rate of 10 to 16 per minute. Local application of atropine restores spontaneous contraction of the ventricle to some extent.

Perfusion of Frog's Heart.—The main results of the perfusion of the separated ventricle by weak doses of indaconitine, are to increase excitability, accelerate the rhythm, and favour the occurrence of group beating. Large doses—0·0004 to 0·0006—produce rapid deterioration in the strength and duration of the systole, but as an early effect, the excitability was increased, spontaneous contractions often appearing after perfusion by indaconitine, whilst no such effect had followed from the use of the simple nutrient fluid. The weakened and rapid beat of indaconitine is rendered slower and stronger when atropine solution is substituted.

Action on Respiration.—After temporary acceleration, slowing of the respiratory movements accompanied by periodical wide inflation of the lungs, follow medium doses of indaconitine, the flank movements being more obviously slowed than those of the hyoid. A proportion of 0·0006 to 0·0008 per kilogramme, whilst having such an effect, does not arrest the respiration, but with a proportion of, and above 0·001 per kilogramme, entire suspension of respiration may ensue, so that when the lethal dose is nearly reached, an absence of respiratory movement (obvious and registrable) may be observed for 24 hours or more. This condition may be coincident with some retention of reflex in the limbs. Before the animal resumes its power of regaining the ventral position when placed on the back, there is some return of respiratory movement.

Hyperlethal doses up to 0·0025 gramme per kilogramme will abolish respiration in from 30′ onward according to their extent. The last evidence of some activity in the respiratory mechanism is elicited by cutaneous stimulation, and especially by placing the frog on the dorsum.

Action on Reflex.—Sensory depression at the periphery is somewhat less powerful in the reflex (brainless) frog, under the influence of indaconitine, than it is under aconitine when equal proportions are given. Otherwise the general character of the effect is similar.

Reaction of Nerve Muscle Preparation.—These experiments were conducted upon brainless frogs, in some, vascular ligature being previously applied to one leg. The dose used was in proportion to the total weight of the frog, irrespective of the parts excluded from the circulation.

Experiments.—*Rana esc.* with brain destroyed received 0·00200 gramme of indaconitine per kilogramme. In five hours, the circulation having completely ceased 90′ previously, the nerve stimulated at 43 cm., the muscle at 16, yielded contraction of the muscle. Maximal stimulation, both direct and indirect, gave a series of fair contractions.

R. temporaria (brain destroyed, left vascular ligature) received the large proportion of 0·024 per kilogramme. In 80′ (heart arrested) minimal excitability, nerve 43, muscle 23 cm.

If after poisoning by a large dose of the alkaloid, the preparation of the nerve be delayed for 24 hours, a great reduction in excitability is witnessed, and the resulting contractions to stimulation are very much diminished in force. Though this statement applies in degree to direct as well as to indirect stimulation, the failure of the nervous intramuscular structures is relatively greater than that of the muscle fibre.

After poisoning by as much as 0·007 to 0·008 gramme per kilogramme, entire absence of all response, even to direct stimulation applied 24 hours later, has been witnessed. Such a result is however exceptional.

In order to test as closely as possible the actual and relative effects of indaconitine and spicaconitine upon nerve and muscle, immersion experiments were employed. Ringer's perfusion solution was the menstruum used, this being well suited to the preservation of activity in nervous as well as in muscular tissue, as demonstrated by the fact that in control experiments in which 20 c.c. of this solution was used for immersing the muscle nerve preparation (the foot being retained as described in 'Phil. Trans.,' B, vol. 195, p. 66), the nerve preserved its excitability for from 45 to 50, and the muscle for 75 to 86 hours. Accurately measured amounts of indaconitine and bikhaconitine solutions were tested on companion nerve-muscle preparations obtained from the same animal, or else one of the preparations was exposed to an aconitine solution whilst the other was used as a control. In all cases the minimal excitability was determined from time to time with as little repetition of stimulation as possible.

With solutions having a proportion of alkaloidal salt of 1/5000 to 1/25000, the effects of indaconitine and bikhaconitine are fairly parallel towards nervous as well as muscular tissue; but with solutions of 1/50000 and up to 1/500000, indaconitine shows a somewhat greater activity relatively towards both structures, though the difference is more marked towards nervous tissue. The most dilute solution of indaconitine which proved directly active upon muscular tissue was 1/800000.

Solutions of 1/1000000 indaconitine (which do not affect muscular tissue) abolish the contractility of the specimen indirectly stimulated four to five hours before parallel solutions of bikhaconitine do so, and even with 1/2000000 the action of indaconitine usually preponderates, though but slightly. In a dilution of 1/2500000 bikhaconitine, the nerve muscle preparation frequently reacts as long as a control preparation to which no addition of an aconitine has been made. The excitability of intramuscular nerve structures is increased by both solutions when of medium attenuation, whilst the production by either of fibrillation is but seldom observed.

The resistance of the nerve muscle preparations in this series taken from frogs very recently obtained, was greater than that of those used in the immersion experiments made with japaconitine and pseudaconitine, upon animals which had been some time in the laboratory. It has not been possible hitherto to institute a thorough comparison of the two series of aconitines under precisely similar conditions, but from the results of a few contrasted experiments, it seems likely that there is no large variation in the activity of the alkaloids under discussion from those previously examined towards muscular and intramuscular motor nervous tissue.

BIKHACONITINE.

Action of Bikhaconitine upon Blood-pressure and Respiration of Anæsthetised Animals (Cats and Rabbits).

The general features of aconitine are reproduced by bikhaconitine with regard to the circulatory system, but the latter develops a stronger action upon respiration, which is slowed and altered in character. Though not so active in this respect as pseudaconitine, it is more so than the other aconitines examined in these researches, and it is to this that the greater toxicity of bikhaconitine is attributable.

In one experiment after the administration of three half lethal doses of bikhaconitine with intervals of 45′, a condition of "delirium cordis" was occasioned by strong vagus stimulation. Irregularity of the heart had already commenced and vagus stimulation (coil 10) had produced a rise of pressure with acceleration of the rhythm by 10 beats per minute, when after a pause of 15″ the secondary coil was approximated to 8 cm., with the above result. The auricle remained in a state of contraction but exhibiting rapid twitchings, the blood-pressure rose for 3″ and then fell rapidly, so that 5″ after the condition commenced the last of the irregular impulses had been recorded. The ventricles showed wild fluctuation apparently in every portion, which persisted for some time after all impulses had ceased in the carotid. No such result has been witnessed hitherto during the numerous experiments performed with the aconitines. Perhaps in this solitary instance it may have been due to the sudden exhaustion of the residue of co-ordinating action left in the vagus mechanism. The injection of atropine after the aconitines has never occasioned this phenomenon.

When the effect of bikhaconitine upon anæsthetised cats and rabbits is contrasted, it appears that the inhibitory action of the stimulated cardiac vagus is sooner abolished in the latter, but that at the time when this occurs section of the vagus still causes increased slowing of the respiration.

Action of Bikhaconitine on Rabbits.

A proportion of 0·00004 gramme per kilogramme (this is slightly less than one-half the lethal) causes acceleration followed by some degree of slowing of the respiration. Salivation rarely occurs. There is no paresis, but the animal is quiet and does not feed for a time. The variation in rectal temperature is not more than 1° C., a slight rise may or may not precede the fall.

0·000065 gramme per kilogramme.—The respiratory changes noted above are accentuated. Free salivation accompanied by chewing and retching movements, some paresis, especially of the hind legs, pupillary dilatation, and decline of temperature by 1·5 to 2° are observed. Recovery takes place in two hours.

0·00008.—As the lethal proportion is approached all the symptoms are exaggerated. After transitory acceleration, there is respiratory slowing to one-quarter or one-fifth of the original. The respiration is for a time dyspnœal, the thorax expands but imperfectly, a prolonged pause in inspiration is usual. Respiration may become strident, and when this is the case dyspnœal spasms may occur. The pupils are dilated. Paresis is considerable, the body becoming limp, this condition extending in degree to the sphincter ani. The fall of temperature amounts from 2 to 3° C., and if the body is not kept covered or exposed to warmth, a reduction of many degrees more is probable and death may result. On one occasion even with such precautions death resulted after a dose of this proportion. Recoveries, however, occur up to 0·0000875 gramme per kilogramme.

0·00001.—This proportion is distinctly hyperlethal, death taking place in about 40′ and being primarily due to respiratory failure. It is preceded by dyspnœal convulsions.

The lethal dose has been determined at 0·0000875 gramme per kilogramme. On one occasion only has a slightly larger proportion been recovered from (0·00009).

From the tables of the effect of readministration of indaconitine and bikhaconitine respectively, as well as from experimental records which are not tabulated, the following points may be indicated:—

1. For both alkaloids there is a marked tolerance when the dose is one-sixth of the lethal administered every 45 or 60′, or even of one-quarter when repetition is not more frequent than hourly.

2. The greatest reduction after such administrations is after the second dose, and thereafter the temperature rises, though showing trifling checks when subsequent administrations are in progress.

3. After smaller, as well as after larger doses, it is peculiarly in those cases in which respiration remains depressed that the relatively late occurrence of the maximum fall of temperature is to be looked for. In fact it may be predicted that the temperature will continue to fall, or at least, fail to rise, so long as respiration remains in the region of 30 to 35 per minute.

Repeated Administration of Bikhaconitine at Stated Intervals to Rabbits. Effect on Temperature and Respiration.

Fraction of lethal proportion per kilo. body weight.	When repeated.	How often.	Total proportion to the lethal.	Greatest fall of temperature.	Subsequent course of temperature.	Note on respiration.
	mins.					
⅙	45	7	1⅙	1°·1	Greatest reduction after second injection.	Respiration varies through 30 per 1′ only.
”	60	6	1·0	0·8	Greatest after second injection.	
¼	45	8	2·0	3·5	Greatest after fifth injection.	Respiration remains slow, 34 to 48 per 1′ during prevalence of low temperature.
”	60	6	1·5	1·2		
½	45	2	1·0	3·2	Greatest after second. A third would have been fatal.	Active dyspnœa induced.
”	90	3	1·5	2·9	Greatest after second injection.	Respiration slowed by 30.
”	120	2	1·0	1·7	Greatest after second.	
”	180	2	1·0	1·7	Greatest after second. The two falls are equal in extent.	Respiration much slowed after each injection.
”	240	2	1·0	1·3	Greatest fall after first injection.	Falls to one-half preexisting rhythm after each injection.

4. When such a proportion as one-quarter of the lethal is administered every 45′, or one-half of the lethal every 45, 60 or 90′, there is a decided summation of effect, and a further, often considerable reduction of temperature ensues upon readministration, so that in the former case the maximal fall does not occur until after the fifth administration.

When a third administration of the half lethal dose of indaconitine is made at intervals of 45′, a lethal issue may occur, though this is exceptional, but the third dose of bikhaconitine so administered is lethal.

5. As between bikhaconitine and indaconitine, it is further observable that the respiration accelerates less rapidly after the repetition of the former than when the latter is given, and to this may frequently be ascribed the greater accession to the fall of temperature after bikhaconitine. It appears that as a result of greater summation of effect produced by bikhaconitine, when doses larger than one-sixth of the lethal are administered, the action of the alkaloid becomes relatively greater than that of indaconitine. The toxicity of bikhaconitine is upon repetition of such doses, greater with regard to indaconitine than the relationship of the unit lethal dose, viz., 0·0000875 gramme to 0·00012 per kilogramme, would indicate.

6. Up to 180′ there is usually summation of bikhaconitine effect after a 0·5 lethal dose, whereas summation after indaconitine for a parallel proportion is rarely witnessed after a longer interval than two hours.

Action of Bikhaconitine towards Frogs (R. temporaria).

(A Synopsis of Experiments is appended.)

0·0009 gramme per kilogramme.—Excitement, then voluntary movement reduced, limb reflexes often uncertain, respiration slowed, ultimately suspended.

0·0012.—Excitement, with frothing on the body. After transitory acceleration, slowing and arrest of respiration, all voluntary movement disappearing, gaping movements, great impairment of reflexes, lasting 4 to 5 days, during which period there is inability to get off the dorsal position.

0·0013.—As above; but cardiac irregularity develops, with ultimate failure of effective systole, the circulation in the web becoming partial, feeble, and intermittent until it ceases altogether. Death takes place in 12 to 14 hours after injection.

Lethal Dose.—In June and July an occasional lethal effect followed a proportion of 0·001 gramme and upwards, until on reaching 0·00125 gramme per kilogramme recoveries were very exceptional. The last may be accepted as the lethal proportion.

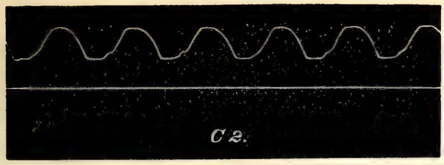
Whilst the action of bikhaconitine is both qualitatively and quantitatively closely similar to that of indaconitine on the heart, the effect of bikhaconitine is greater than that of indaconitine on the frog's respiration.

Action on Respiration.—Suspension of respiration is not essentially inimical to the continuance of life in frogs, and therefore, though bikhaconitine causes an arrest of visible or registrable movement in smaller proportion than indaconitine would, the lethal proportion is practically the same for the two alkaloids. Whilst proportions of 0·0008 to 0·0009 of bikhaconitine may suspend respiratory movement for from 1½ to 2½ hours, doses approaching the lethal prolong the period of inactivity to 36 hours or more.

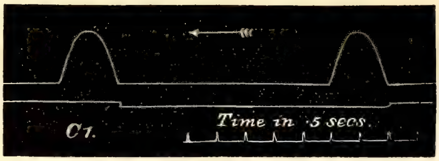
Bikhaconitine upon the Cardiac Rhythm of Frogs.—The effects of this alkaloid are essentially those of indaconitine, as are also the modifications produced in the reaction of the heart to vagus stimulation.

Perfusion of the separated organ has also parallel results. Fig. C_2, C_3 shows the increased excitability, accelerated rhythm, and failing systole of the ventricle under perfusion of relatively powerful solutions of the alkaloid (0·00048 and 0·000549). The two contractions in C_1 are elicited by stimulation before circulation of bikhaconitine.

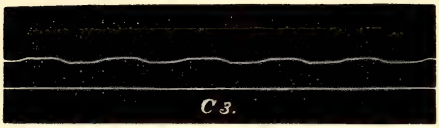
After 0·00048 gramme bikhaconitine.



Perfusion of ventricle with normal solution.



After 0·00064 bikhaconitine.



Reaction of Muscle-nerve from Frogs (R. temp.) *poisoned by Bikhaconitine.*—The results are fairly parallel with those obtained from indaconitine, but the average effect upon the intramuscular nervous tissue seems to be slightly weaker. Experiments *a* and *b* were performed upon brainless frogs; in *b* a vascular ligature was applied to the left leg.

	Dose per kilo.	Heart arrested.	Min. excitability.	
			N.	M.
		mins.	cm.	
a	0·002	200	44	20
b	0·012	110	Lig. 24 Open 30	23 24
c	0·024	75	45	27

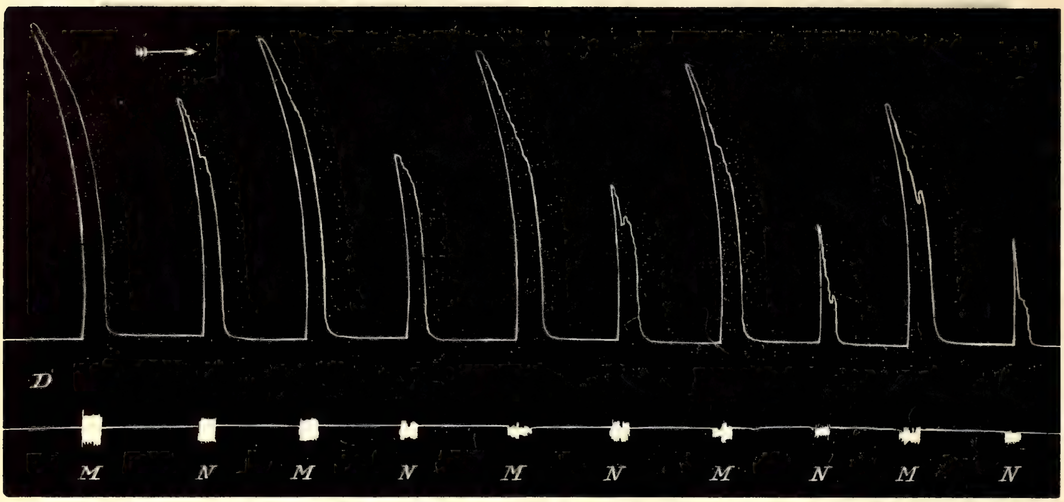
In "*b*," after a series of 10 stimulations (each 3″ faradisation) the contractions of the preparation from the open side showed a decided falling off both in altitude and in maintenance. The subsequent contractions are shown in fig. D: indirect (N) and direct (M) stimulations alternate.

c. In this experiment, though the dose was proportionately larger, less effect was produced at the periphery, owing to the earlier interference with circulation.

The effect of bikhaconitine upon nerve and muscle poisoned *in situ* appears to be slightly less (dose for dose) than is that of indaconitine.

Immersion of Nerve-muscle Preparations in Solutions of Bikhaconitine.—The results of this series of contrast experiments have been given above.

Alternating Nerve Muscle, 3 secs. faradisation. Prepared from frog poisoned by 0·012 gramme bikhaconitine per kilogramme.



Comparison of Pseudaconine from Indaconitine and from Pseudaconitine.

It has been already stated that indaconitine furnishes, as its ultimate hydrolytic product, the same base as pseudaconitine. It was thought desirable to closely compare the physiological action of the pseudaconine from both sources. The amount of material available only sufficed for observation upon frogs: in these animals the lethal dose was determined. Both alkaloids possessed a sweet taste; neither occasioned any of the aconitine effects locally when placed upon the tongue.

Action of Pseudaconine (from Pseudaconitine) in Progressive Doses upon R. temporaria.

0·4 gramme per kilogramme.—No excitement after injecting solution into dorsal sac. Reflexes rapidly disappeared; no spontaneous actions were, as a rule, attempted. In 40′ all reflex had gone and respiration had ceased, except that when placed on dorsum one slight movement of the arms and the respiratory muscles ensued. Heart 24 regular. 2 hours 47′. Reflex beginning to return. Circulation in web moderate, heart 22. Next day quite normal.

1·3 grammes per kilogramme.—As above, though action from larger dose more rapid. Pigment cells distended.

24 hours.—Reflex absent, pupils contracted, circulation moderate.

48 hours.—Sitting up but limp, cannot get off dorsum, tremulous on movement.

52 hours.—Nearly gets off dorsum.

72 hours.—Averse to movement, but hops well if roused, gets off dorsum.

1·7 grammes per kilogramme.—As above, but early symptoms are augmented and accelerated.

24 hours.—Circulation in web feeble and partial.

48 hours.—Circulation improving. Reflexes absent except faint movement of all trunk when placed on back.

96 hours.—Sluggish eye reflex. Faint respiratory movement, gets off back.

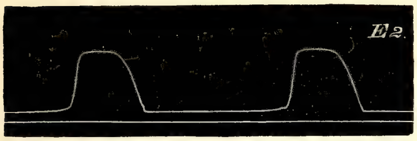
122 hours.—Does not spring yet. Movements rather tremulous.

144 hours.—Hops fairly.

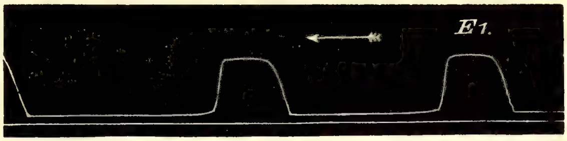
No dose of lower proportion than 1·7 per kilogramme was lethal, whereas larger doses proved so, owing to cardiac failure.

Whilst the lethal dose of this aconine is about 1·75 grammes per kilogramme, its effect in sub-lethal dose appeared to pass off more rapidly than that of pseudaconine from indaconitine. This, however, may have been due to differences in the animals under observation.

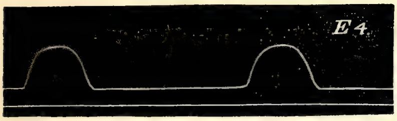
After 0·004 pseudaconine, from pseudaconitine.



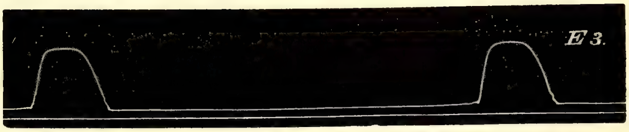
Perfusion of ventricle.

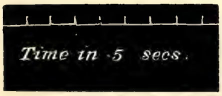


After 0·044 more.

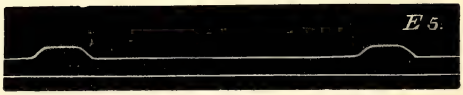


After 0·012 more.





After 0·02 more.



In all 0·08 gramme pseudaconine.

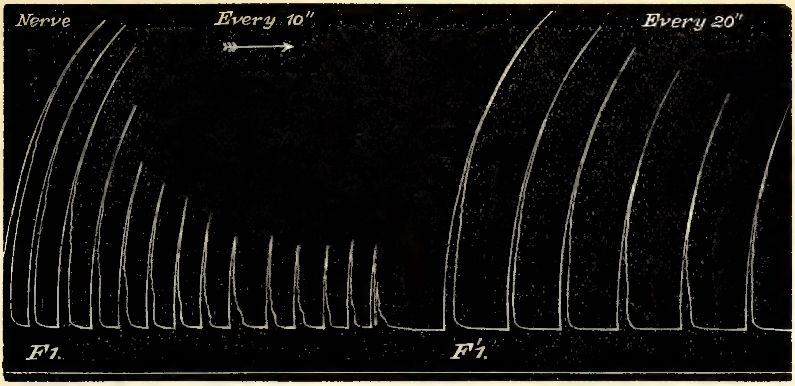
Perfusion of the Heart with Pseudaconine (from Pseudaconitine), Fig. E.—Strengthening of the ventricular systole ensues when a few cubic centimetres of solution (containing 0·02 of alkaloidal salt per 20 c.c. of menstruum) are perfused (E_2), and this effect is still apparent when 20 to 30 c.c. (E_3) have been passed slowly through the organ. When 60 to 70 c.c. have passed, a delay in the systolic phase with retarded relaxation occurs, (E_4), and beyond this amount the systole becomes distinctly feebler (E_5). A spontaneously contracting preparation beats more slowly after small and moderate doses of pseudaconine. Ringer's solution perfused through the organ, which shows evidence of the action of large doses of pseudaconine, is usually sufficient to restore the original strength of contraction.

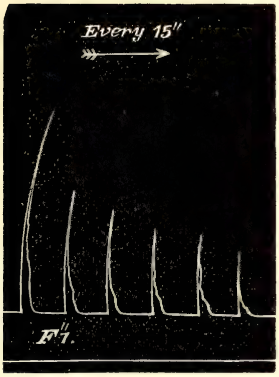
Action on Muscle and Nerve.—When such a proportion of pseudaconine (from pseudaconitine) as 1 gramme per kilogramme is injected into the dorsal sac of a brainless frog in which one sciatic is exposed, it is found that on stimulating the peripheral end of the nerve, the contractions soon become feebler and remain altogether absent, if the stimulation is frequently repeated with but brief rest intervals. After a pause contractions are again elicited, and this phenomenon may be reproduced until under a further and increasing action of the alkaloid, all response to faradisation is eventually lost. According to the dose employed, this absence of response may last for some hours or even for a day or two. The same rapid exhaustion of excitability under stimulation, is witnessed during the earlier phases of recovery of excitability of the intramuscular motor nerves. In the uninjured animal recovering from pseudaconine, the inability to perform a series of spontaneous movements, as well as the rapid failure of the reflexes when a sufficient rest interval is not assured, is due to the same condition. The separated nerve muscle preparation gives the reaction which might be anticipated.

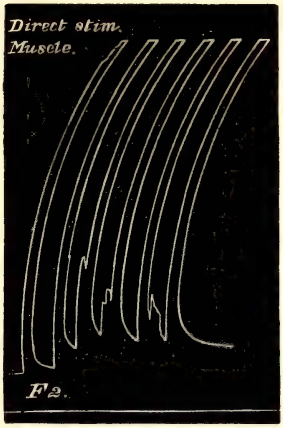
Experiment.—In a pegged frog (*R. temporaria*) a vascular ligature was applied to the left leg, and pseudaconine (from pseudaconitine) was injected under the skin of the abdomen in the proportion of 0·3 gramme per kilogramme. In 45′, when all reflex had disappeared from the open side, two muscle nerve preparations were made, one from either leg, and tested by faradisation. The minimal excitability on the ligatured side was 15 cm. for indirect and 9 for direct stimulation; on the open side 10 and 8 respectively.

The tracings are the result of a series of 3″ faradisations delivered every 10″ in the first series (F_1), every 20″ in the second series (F'_1), and every 15″ in the third series (F''_1). The muscle directly stimulated yields strong and well sustained contractions. This is, therefore, the result of a slight action of pseudaconine, less than one-fifth of the lethal dose having been administered.

Faradisation of nerve-muscle preparation slightly affected by 0·3 per kilogramme pseudaconine from pseudaconitine for 3 secs. on each occasion.







Action of Pseudaconine (from Indaconitine) in Progressive Doses upon R. temporaria.

0·4 gramme per kilogramme.—No excitement after injection. In 36′ all reflex and respiratory movement gone, except that, when placed on the back, one movement of the trunk and protrusion of the hyoid took place. In 2 hours 40′ there is indication of return of reflexes commencing. Next day is normal.

0·8 per kilogramme.—The above symptoms accentuated. In 29 hours there is reflex response in limbs and trunk. Pupil contracted, no distinct eye reflex. In 48 hours, recovered.

1·3 per kilogramme.—In 11′ all reflex gone ; 3 hours, circulation in web is good and general.

24 hours.—No reflex, circulation good, pupil contracted.

48 hours.—Beginning to draw leg up reflexly. Partial eye reflex.

52 hours.—Legs drawn up. Cannot get off dorsum after voluntary effort, ability for further movement is temporarily suspended.

72 hours.—Lethargic, sitting up, hop short, slight tremor on movement.

1·7 per kilogramme.—As above. Circulation in 24 hours is only just moving in larger vessels of web.

48 hours.—Circulation still feeble, but more general; when placed on dorsum, a very slight movement in trunk.

96 hours.—Eye reflex doubtful, but brisk leg reflex when placed on dorsum. Circulation feeble. Respiration is *nil.*

120 hours.—Cannot get off dorsum, but moves spontaneously. Eye reflex present. Faint respiratory movements at long intervals.

144 hours.—Cannot get off dorsum, but crawls if placed on belly. Respiration stronger and more frequent.

168 hours.—Gets off back, still feeble, spring very short.

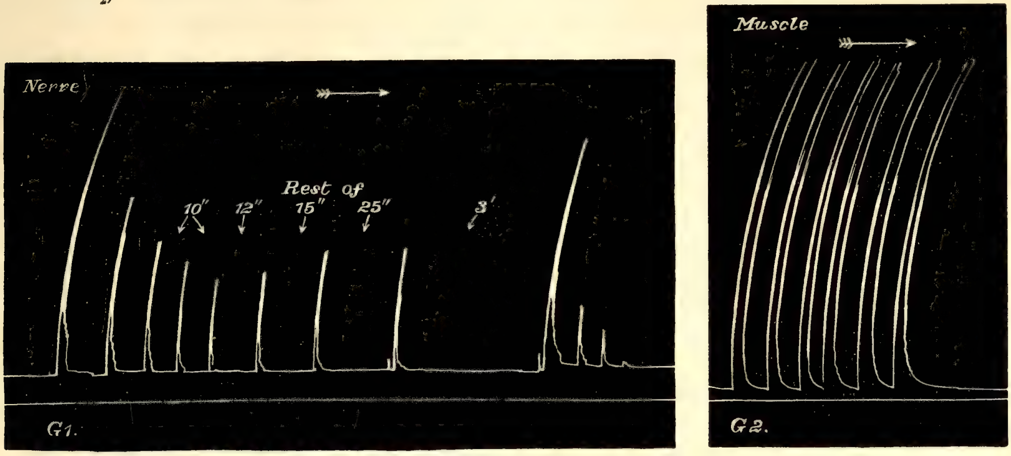
Proportions above 1·7 were lethal from failure of the heart, and so on one occasion was 1·5. It has been impossible, from scarcity of material, to make this estimation more exactly, but it is probable that the toxicity of the two pseudaconines is identical (1·75 gramme per kilogramme), although the duration of action of large doses of the indaconitine product appears to be relatively somewhat longer, a result which may be attributable rather to variations in the animals than to differences in the substances.

Perfusion of the Frog's Heart.—Solutions of pseudaconine (from indaconitine) salt (0·01 in 20 c.c. of menstruum) were found to increase the strength of the systole, and otherwise to occasion the same phenomena as those described for pseudaconine from pseudaconitine. The effect of large doses of the former seemed slightly in excess of that of the latter, but from the nature of the experiment exact contrast is difficult. The excitability of the preparation beating spontaneously so long as perfused by Ringer's solution, seems to decline on substituting pseudaconine solution, spontaneous contraction tending to become less frequent or to disappear. A good contraction is, however, elicited on stimulating.

Action on Muscle-nerve.—The same phenomena are occasioned by both pseudaconines.

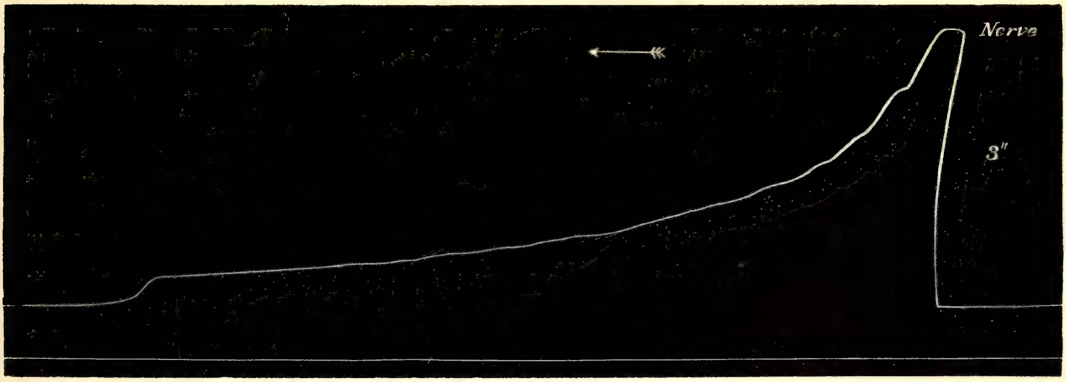
Experiment.—Pegged *R. temporaria,* vascular ligature applied to left leg. Injection 0·3 per kilogramme pseudaconine (from indaconitine). In 20′, and before reflex was quite abolished, two companion nerve-muscle preparations were made. The effect of faradisation (G_1 of nerve and G_2 of muscle) is shown in the figures. Each stimulation is of 3″ duration, and it is observable that the longer the rest interval and the greater the ensuing

G_1, faradisation of nerve-muscle preparation slightly affected by 0·3 gramme per kilogramme pseudaconine (from indaconitine) for 3 secs. on each occasion. The time-interval (marked between stimulations) varies. A pause of 3 secs. before the last group. G_2, direct stim. of muscle.



contraction, the shorter and the fainter the response. There is a rest time of 3′ before the final group is taken. A single response to 3″ faradisation recorded on a rapidly moving surface is shown (fig. H).

H. Single faradisation (3 secs.) of nerve of above recorded on rapidly moving surface.



The section on aconine in a previous paper* may stand for the pseudaconines now under discussion, in so far as the action of these upon reflex excitability of the cord, upon respiration, and upon the form of the muscle curve are concerned. All three appear to possess in common some antagonising action towards the weakening and incoordinating effect produced by the aconitines upon the frog's heart. This circumstance is referred to† when the respective action of aconitine and aconine (from *A. napellus*) was discussed.

---

* 'Phil. Trans.,' B, vol. 190, p. 380.

† *Ibid.*, p. 283.

Summary.

The two aconitines, indaconitine and bikhaconitine, agree in their qualitative effects with the other alkaloids of this series, aconitine, japaconitine, and pseudaconitine, which have been dealt with in our previous papers.

The toxicity of indaconitine is less than that of bikhaconitine towards warm-blooded animals; in this respect the former stands very near to the aconitine of *A. napellus,* whilst the latter being somewhat stronger than japaconitine, is to be referred to a position between this alkaloid and pseudaconitine from forms of *A. ferox* which is much the most active of the series.

The depression of the respiratory function by indaconitine is less than that produced by bikhaconitine, and to this the greater toxicity of the latter is referable. Repeated doses of alkaloids administered at regular intervals and in similar fractional proportions of the lethal dose—are followed by a more marked toxic effect when bikhaconitine is administered rather than indaconitine. Towards frogs the toxicity of the two alkaloids under discussion is practically equal, bikhaconitine is more active than indaconitine in reducing the respiratory activity. On the other hand, it is somewhat less active in abolishing the excitability of muscular and intramuscular motor nervous tissue (immersion experiments), and in reducing the ability of the muscle-nerve preparation poisoned *in situ* for the performance of work sufficient to cause fatigue. The local effect of the two aconitines when applied to the skin by inunction, is equal and similar to that of the aconitines already considered.

Indaconitine and bikhaconitine may therefore be substituted for aconitine and pseudaconitine for internal use, indaconitine being administrable in the same dose as aconitine (from *A. napellus*) and bikhaconitine in proportion of 0·75 of the unit dose of the former, whilst for local application they may be used as contituents of ointments in similar proportions to aconitine.

Pseudaconine from Pseudaconitine and Bikhaconitine.

The action of these is, towards frogs, identical. Their toxicity appears to be practically equal and their effect generally similar to that of aconine (from aconitine). Their action is in the main curari-like in character.

In conclusion it is an agreeable duty to add that many of the observations on temperature, together with control experiments upon the physiological action of the alkaloids discussed in this paper were accurately carried out by Dr. Croll, Second Assistant in the Materia Medica Department of Aberdeen University, whilst the specimens of the pure alkaloidal salts required in the experiments have been prepared in the laboratories of the Imperial Institute by Mr. A. E. Andrews, Salters' Company's Research Fellow.

The Synthesis of a Substance allied to Adrenalin.

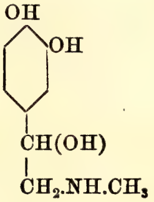
By H. D. Dakin.

(Communicated by Professor E. H. Starling, F.R.S. Received May 29,—Read June 8, 1905.)

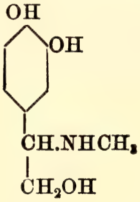
(From the Laboratory of Pathological Chemistry, Lister Institute of Preventive Medicine.)

The isolation of adrenalin from the suprarenal gland by Takamine (1) and by Aldrich (2) in 1901 has led to a considerable amount of work on the chemical nature of this remarkable substance. It is, however, only within the last two years that satisfactory elementary analyses and molecular weight determinations have been made, and the exact structural formula is still unsettled.

Two formulæ have been proposed, either of which will account for most of the observed facts concerning the chemical behaviour of adrenalin, although there are other possible structures which may demand consideration.*



I.



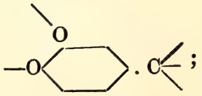
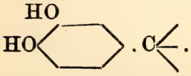
II.

A preliminary note has been published by Friedmann (5), which contains

---

* It is not proposed to give a *résumé* of previous work, but the following indisputable facts upon which speculations as to structure are based may be briefly stated—there is, however, much evidence of a less direct nature which will be found in the papers by Abel (3), von Fürth (4), Friedmann (5), Jowett (6), Pauly (7), and Stolz (8).

(*a*) Adrenalin, on fusion with alkali, gives catechol, so that an ortho-substituted benzene nucleus is present in the molecule.

(*b*) Adrenalin, on methylation and subsequent oxidation, gives veratric acid—3·4 dimethoxybenzoic acid—pointing to the existence of the grouping ; and since further adrenalin gives the colour reactions ($FeCl_3$, etc.) of an ortho-dihydric phenol, it is concluded that two hydroxyl groups are present in the ring .

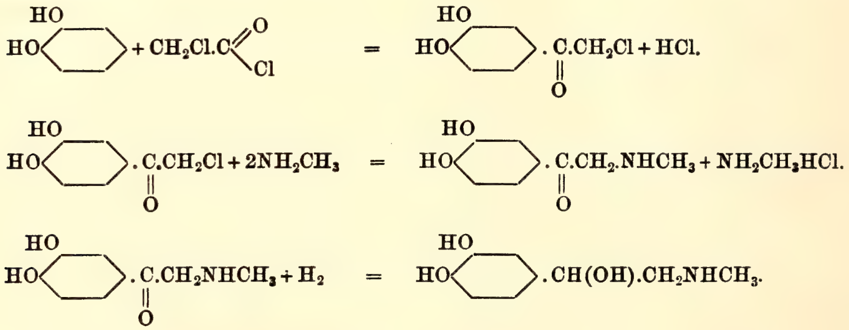
(*c*) On warming with aqueous alkali, methylamine is formed, so that the grouping, —$NH.CH_3$ is present.

(*d*) Adrenalin is optically active, and therefore contains an asymmetric carbon atom which will be situated in the side-chain.

results which would appear to exclude the second formula and support the first. Although criticism must be postponed until further particulars are available, still it may be noted that as at least one of the reactions—the formation of "peradrenalon"—appears to be abnormal, additional experimental evidence is very desirable.*

It is clear that the subject had reached a stage at which synthetical methods might be employed with a possible chance of success, and in January, 1904, I commenced experiments in this direction. A considerable part of my work has, however, been anticipated by workers in the laboratory of Meister Lucius and Brüning (9), and whilst I wish to disclaim any pretensions to priority, I take the opportunity of stating that my results were entirely independently arrived at, and that, owing to the method of publication adopted (Patent Specifications), it is only recently that I have become acquainted with the main portions of their work.

Dziergowski (10) has shown that when catechol is heated with chloracetylchloride, or with a mixture of chloracetic acid and phosphorus oxychloride, chloracetylcatechol is formed. It appeared probable that, by acting upon this substance with methylamine and subsequent reduction of the product, a substance would be produced which should have the formula (I) ascribed to adrenalin:—

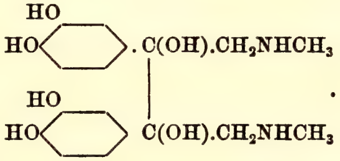


This series of reactions has been carried out, and a substance obtained which has very many of the physiological and chemical properties which are characteristic of adrenalin. It is true, further, that since the synthetic product would necessarily be optically inactive, one would expect slight differences between the artificial and natural bases, but on the other hand there are certain chemical differences, which will be mentioned later, which I am inclined to think are greater than would be the case if the two substances were stereo-isomeric. This involves the conclusion that either adrenalin or the synthetical base has *not* the structure represented by the formula $C_6H_3(OH)_2.CH(OH).CH_2NHCH_3$.

---

* It may not be out of place to note that the statement made in abstracts of Friedmann's paper in the 'Biochemisches Centralblatt' and elsewhere to the effect that adrenalin on oxidation gives adrenalon (methylamino-acetyl-catechol) appear to be incorrect. This would obviously be of the utmost importance if true, but Friedmann apparently oxidised the benzenesulphonic acid derivative of adrenalin, and obtained a substance indistinguishable from the benzenesulphonic acid derivative of adrenalon, which is quite a different matter. As the substances are very badly characterised, too much weight must not be placed on the result, which might possibly be explained in other ways.

A substance which I believe to be identical with the base that I have prepared has been obtained by Meister Lucius and Brüning's chemists by the action of aluminium shavings and mercuric sulphate upon methylamino-acetylcatechol. They definitely ascribe the above formula to the substance, and this, if correct, would necessitate an alteration in the provisional structural formula of natural adrenalin. But it must be remembered that the synthetical base has been obtained by a reaction which is well known to give more than one product. Ketones commonly give a mixture of the corresponding secondary alcohol and pinacone upon reduction, either of which substances may constitute the main product. In the reaction under consideration the pinacone would have the following structure:—



Moreover, it must be remembered that the reaction is carried out, as will be seen later, under conditions which may quite well induce further changes in such unstable substances. Owing to experimental difficulties it is impossible at present to settle the point definitely, but I am of opinion that with the evidence at present available one is not justified in selecting preferentially any one of the several possible formalæ for the synthetical base.

The synthetical substance is extraordinarily active as regards the blood-pressure and certain other physiological effects.

Mr. T. R. Elliott was kind enough to examine some of the substance and informs me that so far as the experiments went they revealed no difference between the stimulating action—both motor and inhibitor—of the laboratory and of the animal products. Such close physiological resemblance may be taken as some evidence in favour of a close structural relationship between the two substances.

Experimental.

Chloracetylcatechol is prepared according to Dziergowski's (10) directions by heating catechol (1 mol.) and chloracetic acid (1 mol.) with phosphorus oxychloride equal in weight to the chloracetic acid used. As the reaction in my hands has always followed a course which differs slightly from that described by Dziergowski, the following particulars are given. The mixture should be placed in a large dry flask—at least 1000 c.c. capacity for 50 grammes catechol—and carefully heated in the water-bath. The mixture rapidly becomes fluid and clear, changing after a time to a deep purple colour. The reaction must be carefully watched, as at a certain point a violent evolution of hydrochloric acid with much frothing is liable to occur, which makes it necessary to cool the flask temporarily. Heating is continued until the evolution of hydrochloric acid slackens, and the thick dark-coloured semi-solid mass is dissolved in about three volumes of hot water. The solution is filtered to remove a trace of oily impurity and then allowed to cool. The chloracetylcatechol crystallises out in very well defined crystals, which are usually somewhat discoloured but are easily purified by a single re-crystallisation from boiling water. The yield is about equal in weight to the catechol taken. The dry substance must be handled with care, as although it is without smell, even minute traces of its dust have a very powerful irritant effect on the eyes and nose.

Methylamino-acetylcatechol.—The first product of the action of methylamine upon chloracetylcatechol is a yellow crystalline methylamine salt ($CH_3.NH_3.O.C_6H_3(OH).CO.CH_2Cl$) (10), but this substance on prolonged digestion with excess of methylamine gives methylamino-acetylcatechol, as has also been shown by Stolz (8). For example, 10 grammes of chloracetylcatechol is placed in a stoppered bottle and 25 c.c. of aqueous 33 per cent. methylamine added in portions of 5 c.c. each. The mixture is cooled between the successive additions. The ketone is almost immediately changed into the yellow crystalline salt, and after thorough shaking and allowing to stand for one or preferably two days, the mixture becomes dark brown from the separation of the crude base. The solid is filtered off and washed well with water, alcohol and ether, then shaken with a slight excess of dilute hydrochloric acid. The solution is filtered from any trace of unchanged chloroketone, concentrated and crystallised. Crystallisation is also economically effected by adding alcohol and subsequently ether to the concentrated aqueous solution, the hydrochloride being precipitated in the form of colourless crystals. The free base may be readily obtained from the hydrochloride by precipitating with ammonia and is, when pure, a colourless crystalline substance, melting at 232° C. and is practically insoluble in water and neutral organic solvents. Most of the salts are readily soluble in water and give a beautifully crystalline compound when shaken with a strong solution of sodium bisulphite. The acetate forms a finely crystalline hydrazone on treatment with phenylhydrazine and a large excess of dilute acetic acid.

Reduction of Methylamino-acetylcatechol.—The reduction offered peculiar difficulties, since the majority of methods applicable for the reduction of ketones were inadmissible owing to the instability of these catechol derivatives in alkaline solution. However, it was found that reduction could be readily carried out electrolytically in acid solution using lead electrodes. The apparatus employed was similar to that devised by Tafel (11), and as it was important to keep the temperature of the liquid in the cathode compartment from rising above 16°, the cell was placed in a freezing mixture.

One part of the base to be reduced is dissolved in the minimum quantity of dilute hydrochloric acid, or preferably the crystalline hydrochloride is taken, and the solution diluted with 15 parts of water. Fifteen parts of 10-per-cent. sulphuric acid are then added to the solution, which is then placed in the cathode compartment. Owing to the sparing solubility of the sulphate of the base, it is advisable to add the sulphuric acid only just before commencing the reduction. The anode compartment is filled with 10-per-cent. sulphuric acid. The reduction is not very rapid, as the time required for reducing 1 gramme of base in a small apparatus, using a current of 3 to 4 ampères, is about half an hour. After the reaction is completed the bulk of the sulphuric acid is removed by adding baryta, and the filtrate contains the sulphate of the new base. A large number of modifications of the above reaction have been tried, but as the products appear to be identical and the above process is the simplest, it is not proposed to give further details, but it may be mentioned that the reduction of the bi-sulphite compound of the base is particularly promising. The aqueous solutions of the salts of the new base, however obtained, may be concentrated *in vacuo*, but are rapidly decomposed if heated on the water-bath.

On addition of ammonia, the free base is thrown out of solution as an amorphous greyish-white precipitate which very readily re-dissolves in acids. The base may be kept suspended in water for some little time without much change, but is so extraordinarily unstable in the dry condition that it has not yet been possible to obtain it in a fit condition for analysis. If one filters the base off as rapidly as possible and quickly removes adherent water by washing with dry alcohol and ether, it is found that the base has already undergone change, and is no longer completely soluble in dilute aqueous acids. This somewhat mysterious behaviour recalls that observed by Victor Meyer (12) in the case of ω-aminoacetophenone ($C_6H_5.C:O.CH_2NH_2$), which readily forms stable salts, but the free base, when precipitated with ammonia, rapidly changes into a non-basic substance. It is mainly this peculiar property which leads one to conclude that it is unlikely that the synthetical base is the racemic modification of adrenalin, for the natural substance is quite stable in the dry state.

The salts of the base show the usual colour reactions with $FeCl_3$, etc., which are common to adrenalin and other catechol derivatives. Potassium ferrocyanide produces no precipitate in the cold, but gives a greenish precipitate on boiling. The free base, like adrenalin, is not precipitated on addition of sodium acetate to solutions of the salts, although under similar conditions the ketone base, methylamino-acetylcatechol, is precipitated. Picric acid produces no precipitate, but silver nitrate is, as one would expect, rapidly reduced.

If a solution of the sulphate be treated with the exact quantity of barium chloride necessary to remove the sulphuric acid and the solution be concentrated *in vacuo* at about 20° to 25°, a syrupy solution of the hydrochloride is obtained, but which shows no inclination to crystallise. The hydrochloride may be partly precipitated as an extremely deliquescent syrupy mass by adding anhydrous ether to a strong alcoholic solution.

The acetate is obtained in similar fashion from the sulphate by means of barium acetate. It has not been obtained crystalline, and seems to be more unstable than the other salts. On adding an alcoholic solution of oxalic acid to the acetate and then precipitating with excess of anhydrous ether, the oxalate is thrown out as a colourless hygroscopic oil which quickly shows distinct signs of crystallising, but it has not been possible to obtain satisfactory analyses for the salt.

A great many attempts were made to prepare stable crystalline derivatives suitable for thorough investigation, but as this end has not been obtained, it is not proposed to give any account of the products obtained by the action of substances such as benzoyl chloride, benzenesulphonic chloride, acetic anhydride, etc., as none of these derivatives were well characterised substances giving satisfactory analytical results.

Effect of Intravenous Injection of the Reduced Base.—The injection of extremely small quantities of the synthetical substance was found to produce a great rise in arterial pressure. A definite rise may be obtained when one millionth of a gramme of the base in the form of hydrochloride is injected into a rabbit with vagi divided. This and other physiological similarities noted by Mr. Elliott would seem to indicate that the difference between the natural and synthetical bases is but slight, and it is perhaps not inconceivable that it is to some decomposition product which may be common to both substances that the stimulating property of the bases is due.

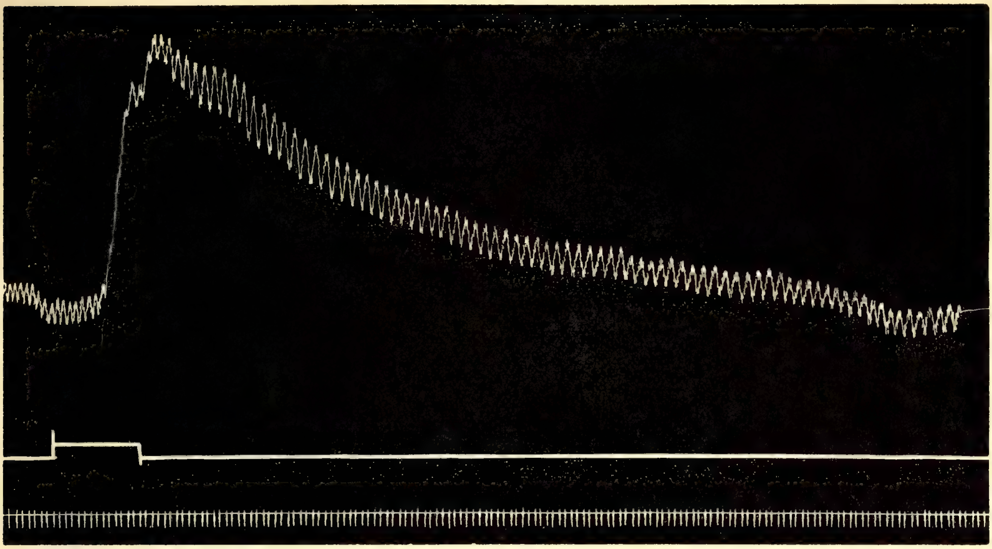


Fig. 1.—Rabbit, 3 kilos. Carotid B. P. Vagi divided. Urethane. Zero pressure 30 mm. below signal line. 0·00002 gramme base as hydrochloride. Time = seconds.

In conclusion I wish to acknowledge my indebtedness to Dr. Leathes for the very large amount of help and advice he has given me throughout the course of the work.

LITERATURE.

(1) Takamine, 'Amer. Journ. Pharmacy,' vol. 73, November, 1901.
(2) Aldrich, 'Amer. Journ. Physiol.,' vol. 5, p. 457, 1901.
(3) Abel, 'Chem. Ber.,' vol. 36, p. 1839, 1903.
(4) von Fürth, 'Sitzungsber. d. K. Akad. d. Wiss. in Wien,' vol. 112, Abt. III, 1903.
(5) Friedmann, 'Beiträge z. Chem. Physiol. u. Path.,' vol. 6, p. 92, 1904.
(6) Jowett, 'Trans. Chem. Soc.,' vol. 85, p. 192, 1904.
(7) Pauly, 'Chem. Ber.,' vol. 36, p. 2944, 1903 ; and vol. 37, pp. 1388 to 1401, 1904.
(8) Stolz, 'Chem. Ber.,' vol. 37, p. 4149, 1904.
(9) D R-P., 157,300, Meister Lucius and Brüning.
(10) Dziergowski, 'Journ. Russian Chem. Soc.,' vol. 25, p. 154.
(11) Tafel, 'Chem. Ber.,' vol. 37, p. 3187, 1904.
(12) Victor Meyer, 'Chem. Ber.,' vol. 28, p. 254, 1895.

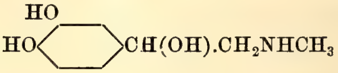
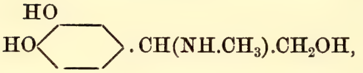
On the Physiological Activity of Substances Indirectly Related to Adrenalin.

By H. D. Dakin.

(Communicated by Professor E. H. Starling, F.R.S. Received May 29,—Read June 8, 1905.)

(From the Laboratory of Pathological Chemistry, Lister Institute of Preventive Medicine.)

Since adrenalin itself possesses such remarkably definite physiological properties which are shared by the synthetical substance described in the preceding paper, it seemed to be of interest to try and trace some connection between their chemical structure and physiological action and, in particular, to see if the activity was to be ascribed to any particular chemical group or combination of groups.

If the formula for adrenalin be either  or  we may artificially divide the molecule into two portions, an aromatic catechol nucleus and an oxyethylmethylamine ($CH_2OH.CH_2NHCH_3$) side-chain. The latter substance was prepared by heating glycolchlorhydrin with methylamine (1) and it was found that injection into a rabbit of quantities up to 10 milligrammes was followed by slight if any rise in blood-pressure. Injections of catechol, on the other hand, produce a great increase in pressure, as may be seen in fig. 1. This fact has also been observed by Mühlmann (2). The amount of substance required to produce an effect is considerable when compared with adrenalin, but a positive result seems significant. It will be seen from the tracing that the effect is prolonged, and it was found that subsequent injection of adrenalin produced no higher retinal pressure than that caused by catechol. When smaller quantities are injected into rabbits it is found that one does not obtain a rapid rise followed by a rapid fall, but both phases are prolonged and the stimulation is submaximal.

The power of causing increased blood-pressure is shared by many other substances containing the catechol nucleus. Thus the two intermediate products in the synthesis of the "adrenalin-like" base described in the preceding paper are both physiologically active. In the case of chloracetylcatechol several milligrammes are required to produce well-marked effects, but methylamino-acetylcatechol is more nearly related to adrenalin, and, as one would expect, is more active physiologically. The properties of this substance have been investigated by Hans Meyer (3) so that it is unnecessary to give further details, but it may be noted that about half a milligramme is necessary to produce a definite rise in blood-pressure in a rabbit, so that the activity of the substance is far inferior to that of adrenalin.

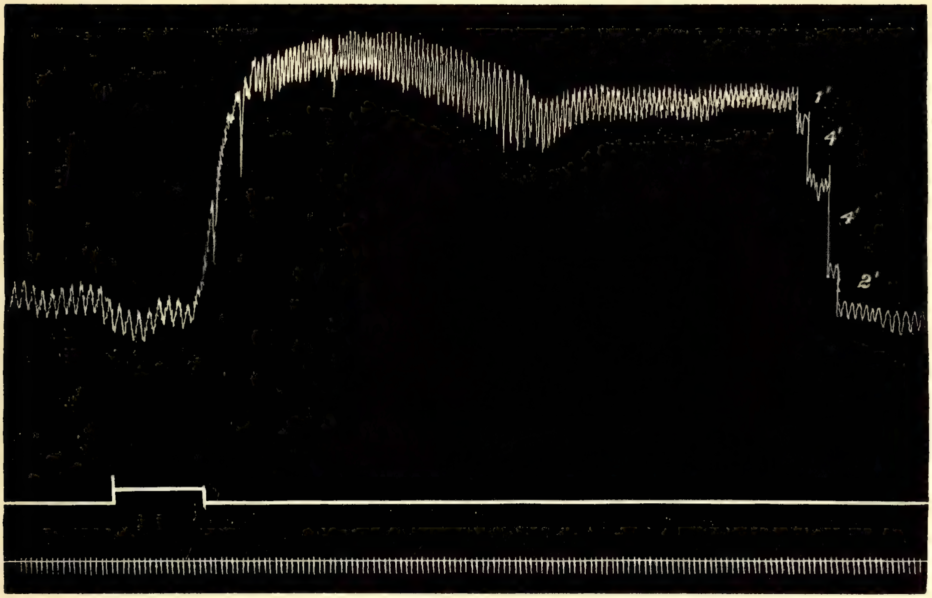


FIG. 1.—Rabbit, 2·2 kilogrammes. Carotid B. P. Vagi divided. Urethane. 10 milligrammes catechol. Zero pressure 30 mm. below signal line. Time = seconds.

If the chlorine in chloracetylcatechol be replaced by hydrogen the product acetylcatechol, $C_6H_3(OH)_2.CO.CH_3$, is still active, but if the hydrogen of the hydroxyl groups be replaced, for example, by acetyl groups the product is quite inactive. Similarly, although, as previously stated, injection of catechol is followed by increase in blood-pressures, the methyl ether of catechol, $CH_3.O.C_6H_4(OH)$, produces no such effect and other analogous cases have been observed. It may be noted that substitution of the hydrogen of the phenolic hydroxyl groups very greatly increases the chemical stability of these substances and this, one may well imagine, would tend to result in substances of less marked physiological activity. From these results it would appear that two free hydroxyl groups in the nucleus are essential constituents of active substances in this group and, since of the three isomeric dihydroxybenzenes only catechol produces a rise in blood-pressure after injection, it is possible that the hydroxyl groups must be in the *ortho* position to one another. Further work is needed, however, before the question can be definitely decided.

As has been already stated, methylamino-acetylcatechol is fairly active in causing increased blood-pressure, whilst the activity of its reduction product is comparable with that of adrenalin. It seemed to be of interest to try the action of the amino- and other alkylamino-acetylcatechols and their reduction products. A considerable difference is noticeable in their physiological properties, corresponding to differences in chemical structure. Thus, amino-acetylcatechol ($C_6H_3(OH)_2.CO.CH_2NH_2$), and the lower alkylamino-acetylcatechols, *e.g.*, the ethyl and di-methyl derivatives, closely resemble the methylamino-acetylcatechol previously described, and their reduction products are very active.

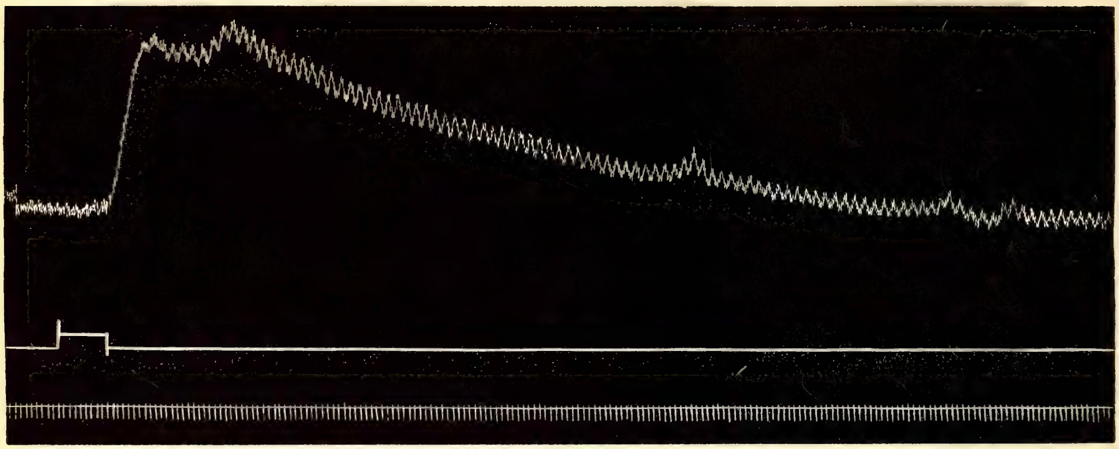
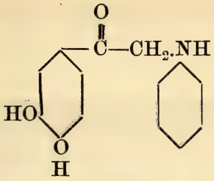


FIG. 2.—Rabbit, 2·5 kilogrammes. Carotid B. P. Vagi divided. Urethane. 0·0010 gramme hydrochloride of ethylamino-acetylcatechol. Zero pressure 30 mm. below signal line. Time = seconds.

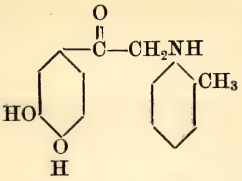
If, however, one takes a higher member of the same series, *e.g.*, heptylamino-acetylcatechol, $C_6H_3(OH)_2.CO.CH_2NH(CH_2)_6.CH_3$, it is found that whilst the ketone base is still active, yet on reduction its activity is but slightly increased.

The base was prepared by the action of excess of heptylamine upon the di-acetyl derivative of chloracetylcatechol, and is a white crystalline substance melting at 125°, and giving beautifully crystalline salts.

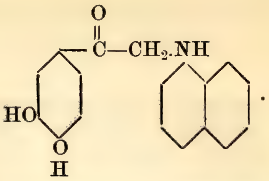
This result shows that the nature of the alkyl group attached to the nitrogen atom is of great importance. If, instead of an aliphatic one substitutes an aromatic group attached to the nitrogen atom the changes in physiological properties are very marked. The following bases were examined:—



Anilino-acetylcatechol (4).



o-Toluidino-acetylcatechol (4).



a-Naphthylamino-acetylcatechol.

None of these bases produced a decided rise in blood-pressure on injection of small quantities. Usually, a fall in pressure was noted, which was least marked with the first substance, and was in this case sometimes followed by a slight rise. This rise was more marked when the substance obtained by its reduction was injected, but even then its activity was far behind the simpler alkylamino derivatives.

As the aromatic bases are only very sparingly soluble in water, they were dissolved in weak alcohol for purposes of injection. Control experiments without the bases were made with satisfactory results. The *α*-naphthylamino-acetylcatechol has not been previously described and was prepared by acting upon chloracetylcatechol (1 mol.) with *a*-naphthylamine (2 mols.) and a little alcohol. It is a faintly yellowish-green coloured crystalline substance, sparingly soluble in dilute spirit and is a very feeble base.

A base ($C_6H_3(OH)_2.CO.CH_2NH.CH_2.C_6H_5$), which may be regarded as intermediate between the two chemical types already described, was obtained by the action of benzylamine upon chloracetylcatechol. The substance is crystalline and readily soluble in alcohol. It had very little effect on the blood-pressure in rabbits, even when injected in fairly large quantities.

Another kind of base was prepared by acting upon chloracetylcatechol with tertiary bases. For example, aqueous tri-methylamine (1 mol.) was digested with chloracetycatechol for some hours, and after adding a drop or two of dilute hydrochloric acid the solution was concentrated and crystallised. Purification is readily carried out by solution in alcohol and precipitation with ether. The substance has the formula $C_6H_3(OH)_2.C = O.CH_2N(CH_3)_3Cl$.

It was found to be *more* active than the corresponding mono-methylamine derivative from which the "adrenalin-like" substance was obtained. In the case of rabbits, so small a quantity as 0·00002 gramme may produce a marked rise. In the comparatively few experiments which were made, it appeared that the substance was very rapidly destroyed after injection, as when quite large quantities were employed the effect was scarcely more prolonged than when the minimal amount necessary to produce maximal stimulation was used.

The reduction of the tri-methylamine derivative did not give products of much increased activity, thus differing in this respect very distinctly from the mono-alkylamine derivatives.

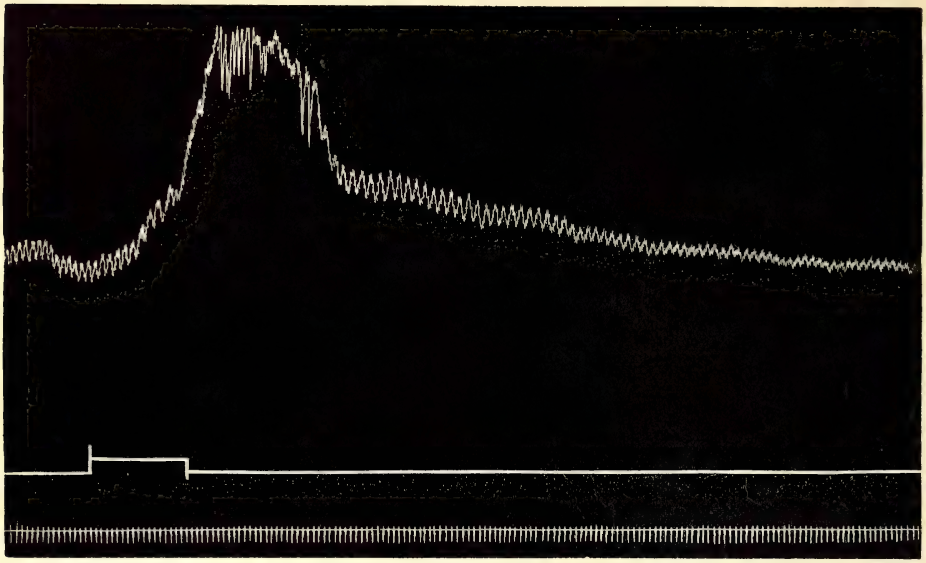
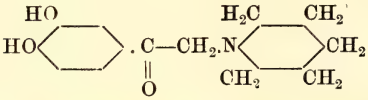


Fig. 3.—Rabbit, 2·5 kilogrammes. Carotid B. P. Vagi divided. Urethane. 0·0005 gramme $C_6H_3(OH)_2.CO.CH_2N(CH_3)_3Cl$. Zero pressure 40 mm. below signal line. Time = seconds.

A corresponding base in the aromatic series was prepared from dimethylaniline and chloracetylcatechol, but, like the other aromatic bases, was not effective in producing rise in blood-pressure.

It is well known that injection of piperidine is followed by increase in blood-pressure (5), and it therefore seemed conceivable that if instead of the ordinary amines used in preparing the bases already described, piperidine was substituted, one might obtain products with increased activity. Experiments, however, showed that this was not the case. Piperidino-acetylcatechol,



, was found to be decidedly less active than the corresponding methylamine derivative, and its activity was scarcely increased upon reduction.

It is obvious that the experiments recorded, although ranging over a number of different types of substances, are very incomplete, yet they seem to warrant certain conclusions, and to indicate the lines upon which one must proceed if one wishes to synthesise substances whose physiological properties more or less closely approximate to those of natural adrenalin.

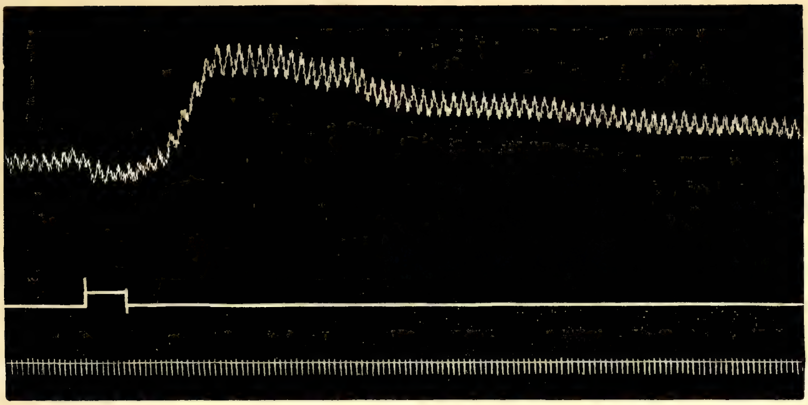


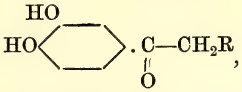
Fig. 4.—Rabbit, 2·5 kilogrammes. Carotid B. P. Vagi divided. Urethane. 0·005 gramme piperidino-acetylcatechol as hydrochloride. Zero pressure 30 mm. below signal line. Time = seconds.

The following deductions are made provisionally, until further experimental evidence is available:—

(i) It appears that the catechol nucleus is essential for the production of physiologically active substances of the type of adrenalin.

(ii) It is of importance that the hydrogen atoms of both hydroxyl groups in the catechol nucleus be unsubstituted.

(iii) An alkyl group of low molecular weight (*e.g.*, methyl, ethyl) attached to the nitrogen tends to produce a much more active substance than when an aromatic group is attached, whilst derivatives of piperidine, heptylamine, and benzylamine occupy an intermediate position.

(iv) The reduction of ketonic bases of the type , where R is a simple aliphatic group, results in the production of bases with enormously increased physiological activity.

(v) In the substances examined there appears to be a connection between chemical instability and physiological activity, and *vice versâ*.

In conclusion I wish to acknowledge my indebtedness to the Research Fund Committee of the Chemical Society for a grant which has partly defrayed the expenses of the work described in this and the preceding paper.

REFERENCES.

(1) Knorr, 'Chem. Berichte,' vol. 22, p. 2088, 1889.
(2) Mühlmann, 'Deutsch. med. Wochenschrift,' No. 26, 1896.
(3) Hans Meyer, International Physiological Congress, Brussels, 1904.
(4) Dziergowski, 'Journ. Russian Chem. Soc.,' vol. 25, p. 154.
(5) Tunnicliffe, 'Centralblatt. f. Physiologie,' vol. 10, p. 777, 1897; Moore, 'Jour Physiol.,' vol. 22, p. 273, 1897.

Transmission and Inoculability of Spirillum Theileri (*Laveran*).

By Dr. A. THEILER.

(Communicated by Colonel David Bruce, R.A.M.C., F.R.S. Received June 28, 1905.)

In 1903 Laveran* described, under the name of *Spirillum Theileri,* a micro-organism which I had found several times in sick cattle. In 1904 I described six cases.† These cases, however, in my opinion did not represent a pure infection of Spirillum, since lesions were present in the red corpuscles, such as basophile granulations, indicating a previous infection with *Piroplasma bigeminum.*

The *rôle* which the Spirillum itself plays as a cause of disease has not as yet been ascertained, but the following notes will help to throw some light on its pathogenic action.

In the communication alluded to above, I described experiments on the inoculation of defibrinated and non-defibrinated blood containing Spirilla from sick into healthy oxen. This experiment failed in eleven animals, although the precaution was taken to utilise, for the most part, imported stock, and the inoculations were made with quantities ranging from five to one hundred cubic centimetres, which were injected subcutaneously, intra-venously, and intra-peritoneally. The injection of blood containing Spirilla into three sheep, two goats, two horses, six rabbits, one guinea-pig, and one rat, also gave negative results. It was then concluded that spirillosis is not inoculable, but this has proved to be a mistake, as further experiments have shown that the disease can be conveyed from sick to healthy animals by the injection of infective blood.

As it has been proved that the Brazilian fowl Spirillum is transmitted by a species of tick, an *Argas,* and since my Spirillum was also found in the blood, the opinion was then expressed, in view of the conclusive evidence of past experiments in tropical piroplasmosis, that cattle spirillosis is also, probably, transmitted by ticks.

The Mode of Conveyance of Spirillosis in Cattle from the Sick to Healthy Animals.—Some observations on bulls on which various kinds of ticks had fed first suggested that this Spirillum might be transmitted by ticks, and, further, that the particular tick in the present case was *Rhipicephalus*

---

* 'Sur la Spirillose des Bovidés,' par A. Laveran.

† 'Comptes Rendus des Séances de l'Académie des Sciences,' vol. 136, p. 939 (Séance du 20 Avril, 1903).

decoloratus. To prove this inference the following experiments were carried out:—

Six heifers, about the same age, which came from Aliwal North, were stabled on the premises of the laboratory, and successfully infected with *Spirillum Theileri* by a tick. In these experiments the tick (*Rhipicephalus decoloratus*) was fed on the sick cattle during and shortly after the febrile reaction, when the Spirillum was noticed in the blood. Spirilla were found to be present in the blood of the healthy animals in some experiments in 13 days, in others in 17 days, after the infected ticks had fed on them. The above experiments thus prove that the *Spirillum Theileri* is conveyed from the sick to the healthy animals by this tick.

The Effect of Injection of Blood from Cattle Suffering from Spirillosis into Cattle, Sheep, Goats, and Horses.—Oxen and sheep were inoculated with blood containing Spirilla from animals suffering from the disease. Spirilla appeared in their blood after two or three days. It is possible that goats can also be infected by the Spirillum, since a slight febrile reaction after inoculation was observed in two cases, but the Spirillum has not yet been demonstrated in the blood of goats. One horse was injected, but proved refractory to spirillosis. The incubation period of the disease, produced by inoculation of infected blood, is as short as two and three days, whilst after tick infection the duration is 13 to 17 days as above noted.

The development of the blue tick from the larva to the adult takes place on the same host. It is necessary, therefore, that the infection of this spirillosis should pass through the egg stage of the tick. When the egg hatches into a larva, the larva is capable of giving the disease to a healthy animal. As the tick remains on the same host for two or three weeks, it is evident that as an adult it may reinfect itself with Spirillar blood, taken from the same animal it infected as a larva. This is possible on account of the short incubation period of the disease. None of the animals died from spirillosis. Enumeration of the red blood corpuscles shows that the Spirillum produces slight anæmia, not sufficient, however, to cause death. Some of the animals went off their feed, and a loss of condition was noticeable.

The Blood of an Ox which has Recovered from Spirillosis is Infective and Blue Ticks fed on such an Animal can acquire the Infection and Transmit it to Healthy Animals.—Five sheep were injected intravenously with defibrinated blood of an immune ox and contracted spirillosis. By an immune ox I mean an animal which has recovered from an attack of spirillosis and is no longer susceptible to the disease. Thus the blood of the healthy immune ox is infective, although it is impossible to determine the presence of the parasite microscopically.

Summary of Conclusions.

1. The *Spirillum Theileri* is naturally transmitted by the progeny of *Rhipicephalus decoloratus* which have developed on cattle suffering from or recovered from Spirillum infection.

2. It is possible to produce spirillosis-susceptible cattle and sheep by the injection of blood from sick or immune animals. The proof that the blood of immune sheep is infective is yet wanting.

3. The pathogenic effect of Spirillum is a slight anæmia accompanied by fever. In none of my cases did a fatal result occur.

An Experimental Enquiry into the Nature of the Substance in Serum which influences Phagocytosis.

By George Dean, M.A., C.M., M.B., Bacteriologist-in-Charge of the Serum Department of the Lister Institute of Preventive Medicine, London.

(Communicated by Professor J. Rose Bradford, F.R.S. Received July 8, 1905.)

Metchnikoff, and his school, in the face of much opposition, lasting many years, have offered convincing proofs of the importance of phagocytosis in the protection of the animal body against bacterial invasion. The main theses of the Metchnikovian theory are now almost universally accepted, but the exact mechanism of the processes involved is even now the subject of keen controversy. If a highly virulent organism is injected into a susceptible animal, the leucocytes appear to be repelled, and to be unable to deal with the microbe, which multiplies and causes the death of the animal. If, however, the suitable immune serum is injected into the animal before inoculation, the phagocytes attack and devour the invading micro-organisms.

Much discussion has centred round the interpretation of such experiments. The early work of Nuttall and others on the bactericidal action of normal serum, and Pfeiffer's demonstration of the bacteriolysis of cholera and typhoid bacilli by immune sera in the absence of cells, formed the chief basis on which rested the humoral theory, which attributed the protection in such cases to the destructive action of the serum on the microbes. Flügge graphically illustrated the view of the humoralists by likening the phagocytes to the trenches made ready behind the fighting line to receive the conquered dead.

It was found, however, that cases of protection resulting from the use of immune serum occurred where no such bacteriolytic action could be demonstrated; the plague bacillus and the streptococcus may be mentioned as examples. Admitting that the phagocyte plays a part in the protection against these infections, the question must still be considered whether the immune serum has acted on the injected microbes or on the phagocytes. Metchnikoff maintains that the serum stimulates the leucocyte to its activity, whereas many workers, who are quite prepared to admit the important part which the phagocyte plays in the process, hold that the immune serum acts chiefly on the micro-organism.

Metchnikoff's view, however, is not opposed to the idea of the immune substance, or "substance sensibilisatrice," becoming fixed on to the cocci. He admits that this occurs, and that the micro-organisms thus sensitised, though they maintain their vitality and virulence, become more readily the prey of the leucocyte, whose activity is increased by the stimulating action of the "substance sensibilisatrice." In the animal body, under normal conditions, bacteriolysis of the microbe occurs within the phagocyte (Bordet and Levaditi). In experiments *in vitro*, or in the animal body where phagolysis has occurred, free cytase or complement being present, bacteriolysis may occur both outside and inside the phagocyte.

It may be of use here to make a brief reference to a few of the investigations carried out by the followers of Metchnikoff with reference to the influence exerted by the serum, on the one hand on the phagocytes, on the other hand on the microbes. The papers selected to illustrate the subject are by Bordet, Savtschenko, and Levaditi, and their views are referred to only in so far as they touch on these points.

Bordet (1895 and 1897) holds that the specific serum contains a thermostable substance, "sensibilisatrice," which acts on the micro-organisms and prepares them for the thermolabile alexin, or proteolytic ferment, which acts as the solvent. He compares the action of the immune serum to that of a mordant. In certain cases, however, such as in streptococcus infection, the bactericidal action is slight, and in such cases he attributes to the immune serum a stimulating action on the leucocytes. The leucocytes and other cells can perceive the presence of a preventive serum, and under its stimulus they are capable of reacting by movement. They manifest towards the immune serum a pronounced positive chemiotaxis. The activity of the leucocytes in the presence of such serum can be observed *in vitro.*

The bactericidal substance is not uniformly distributed through the plasma, but during life is confined within the leucocytes.

Savtschenko and Melkich (1901), from their study of the processes observed in recurrent fever, come to the conclusion that the immune substance, or "fixateur," acts as an intermediary body between the micro-organism and the leucocyte, transforming the negative chemiotaxis of the latter into a positive chemiotaxis. They state that the "fixateur" may act in two ways:—

(1) The leucocytes may absorb the "fixateur," and acquire the chemical affinity necessary to enable phagocytosis to occur.

(2) The "fixateur," which is present in a free state in the plasma, becomes fixed on to the spirilla, to which it communicates the chemical affinity for the protoplasm of the leucocytes, and phagocytosis results.

The latter hypothesis is not invalidated by the fact that no Pfeiffer's phenomenon (of bacteriolysis) can be obtained by supplying alexin to the spirilla, since a much smaller quantity of "fixateur" may be necessary for phagocytosis than for bacteriolysis.

Savtschenko (1902), in a later work, comes to similar conclusions, based chiefly on experiments on the phagocytosis of red blood-corpuscles. The immune substance, or "fixateur," can fix itself on the microbe, or on the leucocyte, and has an affinity for the cytase contained in the leucocyte. It is probable, he thinks, that it acts as a stimuline for the phagocyte. He holds that it acts as an intermediary body between the leucocyte and the microbe, and merits fully Ehrlich's designation of "Zwischenkörper" (intermediary body).

Levaditi (1901) showed, by experiments *in vitro*, that cholera vibrios were ingested by the polynuclear leucocytes of the peritoneal exudate of a normal guinea-pig, and that the intracellular vibrios were converted into granules (intracellular, Pfeiffer's phenomenon), whereas the extracellular organisms remained unaltered. From a series of experiments, he came to the conclusion that this result was due to the presence of "substance sensibilisatrice," in sufficient quantity to enable phagocytosis to occur, the complement for the intracellular change of the vibrios into a globular form being, he believed, supplied from the leucocyte itself.

On the other hand, the extracellular solution of the microbes did not take place owing to a lack of complement. The leucocytic origin of the complement will not be approached in the present paper.

Levaditi also showed that the vibrios on to which the "substance sensibilisatrice" had been fixed when introduced into the circulation of a normal animal were rapidly phagocyted, just as they are in the case of an actively immunised animal. The extraphagocytic conversion of the vibrios into the granular form does not take place if sufficient precautions are observed to avoid injury to the leucocytes.

Denys and Leclef (1895) and Denys (1897) showed that the serum of rabbits immunised against streptococcus had a bactericidal action on the streptococcus, but that the serum of the horse had no such action, though it possessed protective properties. The immune substance in the one case acts as intermediary body between the cocci and the alexin; in the other case between the cocci and the leucocytes.

Denys (1897) made comparative tests of the phagocytic action of different sera *in vitro.* Measured quantities of streptococci were introduced into tubes containing leucocytes, and to certain of these were added immune serum, to others normal serum. By plating loopfuls taken from these tubes, and counting the colonies at various periods, he was able to demonstrate a marked diminution in the tubes containing the immune serum, whereas in the tubes containing the normal serum, an increase was observed.

He found that the leucocyte of the immunised animal was no more active as a phagocyte than the leucocyte of the normal animal. The difference in the two cases was entirely due to a property of the serum.

The conclusion arrived at was that the immunity of the rabbit against the streptococcus was due to a modification of the serum, which rendered phagocytosis possible. The immunity in this case is a humoral property acting by the intervention of the phagocytes.

Mennes (1897), using the same method of experimentation with the pneumococcus, obtained similar results to those obtained with the streptococcus by Denys, and concluded that the immunity in this case was due to a modification, not of the leucocyte, but of the serum. The serum had not acquired any bacteriolytic property, but had itself undergone a change, which resulted in the micro-organisms being taken up and destroyed by the leucocytes.

The results of Denys and also of Mennes are not altogether above the criticism made by Metchnikoff, viz., that the occurrence of a certain amount of agglutination would appear to give a diminution in the number of colonies.

Douglas and Wright (1903), adopting and modifying the method suggested by Leishman, have arrived at a very beautiful technique for the study of phagocytosis, and they have published a series of papers on the subject. The details of the method are too elaborate for reproduction here, but the essential point consists in enumerating the bacteria ingested in a number of polymorphonuclear leucocytes, and, by division, obtaining an average, which is taken as the measure of the phagocytic power of the blood. They find that there is present in the normal blood serum a substance which prepares the bacilli, so that they are capable of ingestion by the phagocytes. They call this effect an "opsonic" effect ("Opsono," "I cater for, I prepare victuals for"), and they use the term "opsonin" to designate the element in the blood-fluids which produces the effect. They find that the "opsonin" is a thermolabile body, *i.e.*, is destroyed by heating to 60° C. for 10 minutes. The leucocyte is an indifferent factor in the matter.

As a result of inoculation with bacterial vaccines, the amount of the opsonin present in the blood may be increased.

Bulloch and Atkin (1905) have confirmed and extended these results of Wright and Douglas. They find that the "opsonin" disappears from serum when the latter is mixed with bacteria at 37° C. or 0° C. The action of heat is to destroy the "opsonin," and not merely to convert it into a "non-opsonisable" modification. It is a simple body, and is not identical with any of the antibodies hitherto discovered in the serum. Leishman, at a discussion on the subject at the Pathological Society, London, supported the "stimuline" view of the action of serum.

Neufeld and Rimpau (1904) find that the immune sera, in the case of the streptococcus and pneumococcus do not stimulate the leucocyte in Metchnikoff's sense, but act on and change the micro-organisms, so that they are secondarily taken up by the phagocytes. They find that the substance which produces this effect is thermostable. A polemic has been waged with Wright as to the identity of this body with the "opsonin" (*cf.* Savtschenko and Markl).*

Hektoen and Ruediger (1905) employed the method of Wright and Douglas, and confirm most of their results. They conclude that the "opsonin" has a complex constitution, there being present a haptophorous group, which fixes on the microbe, and an opsinophorous group, which produces a physical or chemical change in the microbe.

Introduction to Experiments.

The present investigation was undertaken with the view of studying certain questions as to the relation between the phagocytic immunity which occurs in normal animals and in those which have been actively immunised. The serum of a number of animals, chiefly horses, which have been immunised against various microbes, was examined and compared with the serum of normal animals of the same species. At first the method employed was Wright and Douglas', to which reference has already been made.

To save repetition, it may be stated that in almost every case equal volumes of washed leucocytes, of the serum and of the bacterial emulsion were employed. The number of ingested cocci were enumerated in 22 polymorphonuclear leucocytes, and the average taken as the phagocytic index.

---

* The writer had observed the thermostability of the substance in Staphylococcus immune serum and in normal serum before hearing of the work of Neufeld and Rimpau. Where the term "the substance" is employed in this paper, it is used as an abbreviation for "the substance which prepares the micro-organisms for phagocytosis."

Estimations were made of the phagocytosis in the case of animals immunised against *Staphylococcus pyogenes aureus,** *Streptococcus pyogenes*, *B. typhosus*, and *B. dysenteriæ.*

These experiments led to a closer study of the nature of the substance in serum which assists in the process of phagocytosis; and to some extent this enquiry resolved itself into a study of the relations which the immune substance, amboceptor, substance sensibilisatrice, or fixateur, bears to the opsonin of Wright and Douglas. For this investigation the *Staphylococcus pyogenes aureus* was chiefly employed owing to the convenience with which emulsions, counts, etc., could be made. Two races of the Staphylococcus were used, one highly virulent, recently isolated from the human body, the other an old laboratory culture. No marked differences were observed in the counts obtained from these.

Where the method of Wright and Douglas has been rigidly employed, the results I have obtained have been almost entirely in agreement with theirs. I should not feel disposed, however, to place quite the same reliance as they do on the numerical accuracy of the results which can be derived from the method. Where the leucocytes are very full—*i.e.*, where the counts are high—it is impossible to differentiate results by the method of enumeration. In this paper differences obtained by all refinements of enumeration have been neglected. In all cases an excess of cocci was present. The majority of the counts have been made by two or three observers who did not know the objects in view, so that personal bias was eliminated. Without entering into details, it may briefly be stated that, when using their method, my experiments tend to confirm theirs in that there is in normal serum a substance which prepares the microbe for the leucocyte; that this substance, or "opsonin," as tested by Wright's method, appears to be destroyed at a temperature of 60° C. for 15 minutes, and that the leucocyte is, in a certain sense, an indifferent factor. By the employment of other methods, results in oppositition to Wright and Douglas' have been obtained.

When, however, the question of the thermolability of the "opsonin" was investigated in another manner, the results obtained were opposed to the view that the substance was destroyed at the temperatures named by Wright. Indeed, it was found that the heated immune sera, even on testing by Wright's method, gave high phagocytic indices. These results suggested a re-investigation of the effects of temperature by other methods.

---

* Referred to hereinafter as "Staphylococcus."

One method employed was that of adding a measured quantity of a microbial emulsion to a certain volume of serum which had been heated to 60° for 20 minutes, allowing this to stand for varying periods at 37° C., then centrifugalising and ascertaining whether the centrifugalised cocci were capable of being taken up by the leucocytes.

Experiment.—0·5 c.c. of an emulsion of Staphylococcus (= $\frac{1}{2}$ agar tube) was added to 5 c.c., of a normal horse serum, which had been heated to 60° C. for 20 minutes. The cocci, separated by centrifugalisation and made into a suitable emulsion with normal salt solution, were compared with normal fresh cocci in Wright's tubes.

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Emulsion of fresh cocci	1	4
+ Heated normal horse serum	1	
+ Normal horse leucocytes	1	
Emulsion of prepared cocci	1	60
+ Normal salt solution	1	
+ Normal horse leucocytes	1	

Experiment.—0·1 c.c. of an emulsion of Staphylococcus (= 1/10 of an agar tube) was mixed with 1 c.c. of normal human serum, which had been heated to 60° C. for 20 minutes. This mixture was placed for 15 minutes at 37° C., and then the cocci were centrifugalised from the mixture and made into a suitable emulsion with normal salt solution.

These prepared cocci and fresh cocci were used for a comparative test in capillary tubes in the ordinary manner.

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Fresh cocci	1	0
+ Heated normal serum	1	
+ Leucocytes (human)	1	
Prepared cocci	1	60
+ Normal salt solution	1	
+ Leucocytes (human)	1	

A large number of similar experiments which need not be detailed here were done with concordant results; in several cases a smaller proportion of serum to bacterial emulsion was used.

Such results were obtained in the cases of normal human serum (four samples), horse serum, goat serum, rabbit serum, guinea-pig, and rat serum. Normal horse serum which had been kept for four years still retained this property.

These experiments prove that though the results obtained by using Wright's method seem to demonstrate that the substance capable of preparing the microbes for phagocytosis is destroyed by heating for 20 minutes in 60° C., only a fractional destruction of the substance occurs. The loss in the case of sera, such as normal sera, which contain only a comparatively small quantity of the substance, is so great that a method where very small quantities are used makes the demonstration and estimation of the substance impossible.

On the other hand, where a large amount of the substance is present, as in certain immune sera, the heating to 60° C. for 20 minutes, or for even much longer periods, leaves enough of the substance undestroyed to give results by Wright's method. Indeed, the counts obtained may be higher than with fresh normal serum of the same species.

One or two experiments may be quoted as examples of a large number done which gave consistent results :—

Experiment.—

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Fresh normal rabbit's serum	1	9
+ Suspension of Staphylococcus	1	
+ Leucocytes of horse	1	
Heated normal rabbit's serum	1	0·2
+ Suspension of Staphylococcus	1	
+ Leucocytes of horse	1	
Immune rabbit's serum (heated to 60° C. for 20 minutes)	1	20
+ Suspension of Staphylococcus	1	
+ Leucocytes of horse	1	

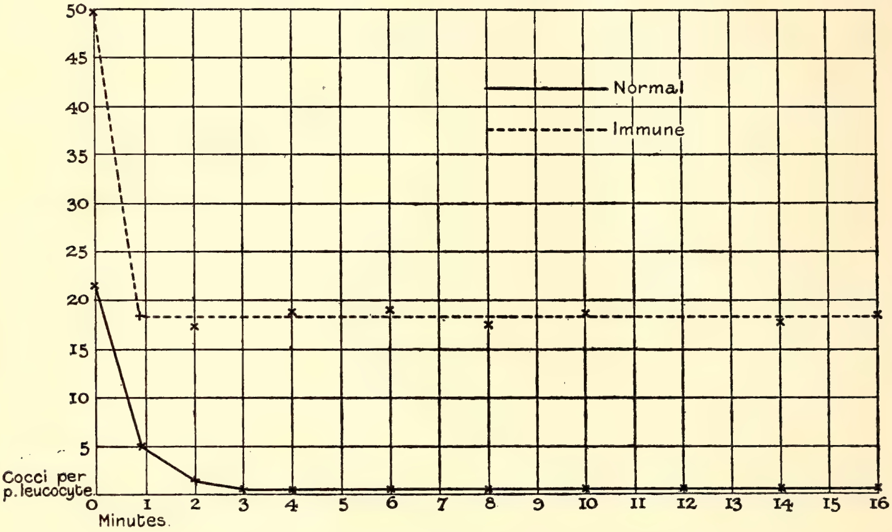
The serum of the normal horse, even when heated to 60° C. for 20 minutes, gave in certain cases, by Wright's method, fairly high counts. It contained a larger quantity of the substance than heated normal rabbit's serum.

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Fresh normal horse serum	1	30
+ Suspension of Staphylococcus	1	
+ Leucocytes of horse	1	
Fresh normal horse serum (heated to 60° C. for 20 minutes)	1	8
+ Suspension of Staphylococcus	1	
+ Leucocytes of horse	1	
Immune heated serum of horse	1	60+
+ Suspension of Staphylococcus	1	
+ Leucocytes of horse	1	

These experiments show that in an immune Staphylococcic serum enough of the substance remains undestroyed by heating to be demonstrable by the use of Wright's method.

When fresh serum, either normal or immune, is heated to 60° C. for various periods, one finds that, as estimated by Wright and Douglas' method, there is a great fall in the first two minutes, and after that the curves run almost parallel to the base line. In the case of normal serum the fall is so great that the curve may reach the base line.

The accompanying chart gives a graphic representation of what happened on heating a normal rabbit's serum and an immune Staphylococcus rabbit's serum for different periods up to 16 minutes. The number 50 applied to the original strength of the immune serum is only approximate, as in such high counts accuracy is impossible. It will be seen that after one minute's heating the immune serum fell to about 18 cocci per leucocyte, and, allowing for experimental errors (the average of a large number of counts was taken), it then runs parallel to the base line.



The normal serum fell from 20 to 5 at the end of the first minute, to 1 at the end of the second minute, and then ran almost parallel to the base line, the average phagocytic index of a number of counts being about 0·5.

The results after 30 minutes showed that no great change had occurred.

One must remember that Wright and Douglas' method probably demonstrates the presence of the "opsonin" over only a very short range. The brief time during which the substance is allowed to act on the cocci probably admits of only fairly high concentrations being indicated. If one compares what occurs in the case of agglutination, only the very strongest agglutinating sera would be regarded as giving positive results if 15 minutes were put as the limit of time for the serum to act on the microbes.

As has been shown by the experiments quoted, the fall in the curve does not indicate complete destruction of the substance, as was stated by Bulloch and Atkin, but merely indicates that the substance has reached a concentration below that demonstrable by Wright and Douglas' method.

This view is emphasised by the experiment detailed in the following section.

Effect of Continued Heating on the Substance in Serum.

Two series were prepared with normal horse serum heated at 60° C. for different periods. In each tube 1 c.c. of the heated serum was placed, and to the tubes of the one series 0·08 c.c., to those of the other 0·05 c.c., of a coccal emulsion was added. The mixtures in each case were put for 15 minutes at 37°, then centrifugalised, and the centrifugalised cocci tested in the usual way. In most cases the leucocytes had to be noted simply as full, but where an obvious fall in the number of ingested cocci was observed, an enumeration was made.

Time for which serum was heated at 60° C.	Tubes to which 0·08 c.c. coccal emulsion added. No. of cocci per leucocyte.	Tubes to which 0·05 c.c. coccal emulsion added. No. of cocci per leucocyte.
20 minutes	Full	Full
40 „	Full	Full
60 „	Full	Full
2 hours	36	Full
3 „	30	44
4 „	30	21

This experiment proves that normal horse serum after being heated for four hours at 60° C. still contains enough of the substance to prepare a large number of the cocci for phagocytosis. With the quantities of coccal emulsion employed in this experiment a fall was observable with the larger quantity only after two hours' and with the smaller quantity after three hours' heating.

On the Influence of Temperature on the Rate of Combination between the Substance and the Cocci.

0·1 c.c. of an emulsion of Staphylococcus was added to 2 c.c. of normal human serum, which had been heated to 60° C. for 25 minutes. The emulsion was at once divided into two parts, and one part placed at 37° C., and the other at about 6—8° C., in the ice-chest. At the end of half an hour they were centrifugalised, the centrifuge buckets having been cooled down. The centrifugalised cocci in the two cases were then tested.

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Coccal emulsion which had been kept at 37° C.	1	22
+ Human leucocytes	1	
Coccal emulsion which had been kept at 6—8° C.	1	2·2
+ Human leucocytes	1	

This experiment seems to show that the substance acts on or becomes attached to the cocci much more slowly at low than at high temperatures.

Microbes which have been placed in Contact with Immune Serum are capable of taking up an Excess of the Substance which can again Diffuse into the Surrounding Fluid.

0·5 c.c. of an emulsion of Staphylococcus was put in contact with 2 c.c. of a heated immune serum and left in contact at 37° C. for 1 hour. The cocci were then separated by centrifugalisation, washed and put into 1 c.c. of an immune serum, X, and left in contact for an hour at 37° C., then centrifugalised very carefully so that all the cocci were thrown down.

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Fresh serum "X"	1	37
+ Staphylococcic emulsion	1	
+ Washed horse leucocytes	1	
Serum, X, through which prepared cocci have been centrifugalised	1	48
+ Staphylococcic emulsion	1	
+ Washed horse leucocytes	1	

This points to a diffusion of the substance into the serum from the cocci.

Experiment.—Cocci which had been treated, as in the previous experiment, with immune serum, after being centrifugalised and rapidly washed with normal salt solution, were left in contact for 24 hours with normal salt solution, which was then tested for the presence of "opsonin."

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Washed leucocytes of horse	1	0·5
+ Normal salt solution	1	
Washed leucocytes of horse	1	21
+ Normal salt solution, from above prepared cocci	1	

A number of experiments were carried out with the view of ascertaining the relation of the substance in normal serum to the substance in immune serum.

Experiment to ascertain whether Cocci fully occupied by the Substance from Normal Serum are capable of absorbing the Substance from Immune Serum.

An emulsion of Staphylococcus, prepared in the usual manner, was added to normal horse serum which had been heated for 25 minutes to 60° C. The mixture was placed at 37° C. for 15 minutes, and the cocci were then centrifugalised till all were removed from the fluid. The supernatant fluid was then removed and the cocci rapidly washed with normal salt solution and again thrown down, great care being taken to avoid any loss of the cocci during the process.

To equal parts of a certain Staphylococcus immune serum from the horse, also heated to 60° C. for 20 minutes, there were added equal parts of the sensitised cocci and of fresh normal cocci. These mixtures were then centrifugalised, and the phagocytic indices of the supernatant fluids estimated and compared, the usual proportions of washed leucocytes and Staphylococcus emulsion being used.

	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Original heated immune serum	24
Original heated immune serum through which prepared cocci had been centrifugalised	13
Original heated immune serum through which normal cocci had been centrifugalised	4

These numbers are the mean of three counts each of 22 polymorphonuclear leucocytes.

A similar experiment carried out with a dilution of one in five of a similar serum of greater potency gave the following numbers:—

	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Phagocytic index of the dilution of original Staphylococcic immune serum of horse	30
Ditto through which prepared cocci had been passed	24·3
„ „ normal „ „	8

These two experiments seem to show that the cocci occupied by the substance from normal serum are incapable of taking up the substance from immune serum, whereas fresh cocci are capable of removing a large proportion of the substance.

The converse experiment was carried out. In this case the cocci were first prepared by contact with immune serum and then passed through a normal serum. For convenience of estimation by Wright's method, instead of a normal heated serum a normal fresh unheated serum was employed.

Equal measured quantities of fresh cocci and of cocci prepared as in the previous experiments by contact with heated immune serum of the horse were added to equal volumes of normal horse serum, centrifugalised, and the supernatant fluid, which was then free from all organisms, tested for phagocytic power, normal washed horse leucocytes being used.

	Average number of cocci per leucocyte in 22 polymorphonuclear leucocytes.
Original serum	40
Serum through which prepared cocci had been passed	30
Serum through which fresh normal cocci had been passed	2·3

The repetition of this experiment with freshly prepared material gave the following numbers :—

	Average number of cocci per leucocyte in 22 polymorphonuclear leucocytes.
Freshly prepared serum	35+
Ditto through which the prepared cocci had been passed	27
Ditto through which fresh normal cocci had been passed	2

In carrying out these experiments care must be taken that excess of substance is not left adhering to the cocci, in which case it may diffuse out into the surrounding fluid. See previous experiments. To obviate this happening the prepared cocci must be rapidly washed in normal salt solution.

These experiments seem to show that the cocci occupied by the substance from immune serum are incapable of taking up much of the substance from normal serum, whereas fresh cocci are capable of removing a large proportion of the substance.

Tests of the Serum of Animals Immunised against the Streptococcus, Typhoid and Dysentery Bacilli.

Streptococcus.

The serum of three horses immunised against many races of streptococci was examined with the following result :—

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Horse 1—		
Washed leucocytes of horse	1	9
+ Serum of immune horse	1	
+ Emulsion of streptococcus from agar	1	
Washed leucocytes of horse	1	4
+ Serum of normal horse	1	
+ Emulsion of streptococcus from agar	1	
Horse 2—		
Washed leucocytes of horse serum	1	12
+ Serum of immune horse	1	
+ Emulsion of streptococcus from agar	1	

	Vol.	Cocci counted in 22 leucocytes. No. of ingested cocci per leucocyte.
Horse 2—*continued.*		
Washed leucocytes of horse serum	1	3
+ Serum of normal horse	1	
+ Emulsion of streptococcus from agar	1	
Horse 3—		
Washed leucocytes of horse serum	1	23
+ Serum of Horse 3	1	
+ Emulsion of streptococcus from agar	1	
Washed leucocytes of horse serum	1	4
+ Serum of normal horse	1	
+ Emulsion of streptococcus from agar	1	

Typhoid Bacillus.

	Vol.	Bacilli contained in 22 polymorphonuclear leucocytes. No. of ingested bacilli per leucocyte.
Serum of normal horse heated to 60° C. for 20 minutes	1	22
+ Washed horse leucocytes	1	
+ Emulsion of typhoid bacillus	1	
Serum of immune horse heated to 60° C. for 20 minutes	1	49
+ Washed horse leucocytes	1	
+ Emulsion of typhoid bacillus	1	

Dysentery Bacillus (Shiga).

Serum of normal horse heated to 60° C. for 20 minutes	1	1·5
+ Washed horse leucocytes	1	
+ Emulsion of dysentery bacillus	1	
Serum of immune horse heated to 60° C. for 20 minutes	1	25
+ Washed horse leucocytes	1	
+ Emulsion of dysentery bacillus	1	

In the case of the typhoid and dysentery bacilli considerable difficulty was found in estimating the phagocytosis on account of the agglutination. The leucocytes, crammed full of bacilli, were found in some cases lying in groups close to bacillary clumps, in other cases it seemed as if the leucocytes when full of microbes tended themselves to become agglutinated.

It is possible that an agglutination of the bacilli towards the leucocytes may be a part of the process which enables the leucocyte by the movements of its own protoplasm to englobe the microbe. The extraordinary rapidity with which a bacterial field is cleared of micro-organisms suggests such an occurrence.

An interesting observation was made in this connection, viz., that organisms after being stained with fuchsin, which has a strong agglutinating action, were capable of ingestion, whereas the same organisms killed by heat were refused by the leucocyte.

It was observed that the normal serum of the same horse heated to 60° C. for 20 minutes had a considerable "opsonising" action on the typhoid bacillus, whereas it had little effect on the dysentery bacillus. Twenty-two typhoid bacilli were ingested per leucocyte compared with 1·5 dysentery bacilli.

Summary and Remarks.

An immune staphylococcic serum obtained from the rabbit when heated to 60° C. still contains a substance capable of preparing the cocci for phagocytosis. An identical result was obtained in the case of an immune staphylococcic serum from the horse. Efforts were made to ascertain whether this substance was identical with the substance in normal serum giving rise to the same effect, *i.e.*, to the "opsonin" of Wright. It was found that when the cocci were added to a fairly large volume of normal serum which had been heated to 60° C. for 20 minutes, incubated for 15 minutes and centrifugalised, they were rapidly phagocyted.

It appeared, therefore, that the heating to 60° C. had produced only a fractional destruction of the opsonin, which in the case of normal serum was present in such small amount that it was no longer measurable by Wright and Douglas' method.

This view was confirmed by heating for various periods both normal and immune serum and comparing the fall resulting. The curves obtained in both cases were similar. The serum of various animals, the goat, rabbit, horse, guinea-pig, rat, and human were tested for the thermostability of the substance. In all it was found to be thermostable when tested in the way mentioned, but more of it appeared to be present in the serum of the horse than in the serum of, *e.g.*, man and rat, since in the case of the horse it was found to be present, after heating, in an amount which was still capable of being demonstrated by Wright's method. Horse serum heated for four hours to 60° C. still contained a large amount of the substance.

Experiments were carried out with the view of ascertaining the relation of the substance in normal serum to the substance in immune serum.

Cocci which had been prepared by contact with normal serum so that they were probably fully occupied by the substance were passed through heated immune serum. On removal of the cocci by the centrifuge the supernatant fluid, when tested with fresh cocci, was found to have lost little, or none, of its original strength; whereas the same fluid through which fresh cocci had been centrifuged had lost practically all its power. The converse experiment gave a quite similar result. In this case, however, care must be taken to avoid the cocci being overloaded with immune serum, in which case they are capable of giving off some of their substance into the suspending fluid.

In this case also it is more convenient to use unheated normal serum, which enables one to easily estimate the opsonic power by Wright and Douglas' method. These two groups of experiments are strongly in favour of the substance in normal serum being identical with the substance in immune serum.

The relation which the "opsonin" of Wright and Douglas bears to the "immune substance," or "fixateur" in so far as the latter influences phagocytosis, as shown by Savtschenko among others, must be briefly discussed.

If the "opsonin" of normal serum were completely destroyed by heating to 60° C. for 15 minutes we should be compelled to assume that it was a separate and new body, and that the increase in the serum of the property of preparing the microbes for phagocytosis, which results from the injection of bacilli, was due to an entirely different body, since the substance resulting from such bacterial injections is markedly thermostable, even when tested by Wright and Douglas' own method.

The experiments which are recorded in this paper, however, show that the destruction by heating of the "opsonin," even of normal serum, is only fractional, and that its apparently complete disappearance is due to the method of observation employed, which demonstrates its presence over a very short range. According to the ordinary use of the word in such investigations the body is thermostable.

The fact that this specific substance is present in small amount in normal serum is in accord with the numerous observations of the occurrence of immune substance in normal sera. One need only refer to the normal antitoxin (*e.g.*, of diphtheria), anti-ferments, etc., and to the fact that the bacteriolytic and hæmolytic actions of normal serum are due to the presence in the serum of an immune substance plus a complement, as has been firmly established by the work of Pfeiffer, Bordet, Moxter, Ehrlich and Morgenroth, and others. In giving the name of "opsonin" to the substance which becomes attached to the micro-organisms and prepares them for phagocytosis, Douglas and Wright have, therefore, named a property of serum which had already been recognised by a number of different workers.

Whether free complement may take part in the preparation of the microbe is difficult to determine. From the experiments detailed in this paper it is certain that it is not a necessary participant in this action. At the same time it is not improbable that the immune body when aided by complement may act more powerfully, and that the sudden fall in the "opsonic" power of both normal and immune serum on heating is due to the destruction of the complement. I may revert to this subject on another occasion.

Metchnikoff's statement in regard to natural immunity, that the leucocytes undertake the struggle against the microbes and free the organisms from them without the need of previous help on the part of the humors, is apparently largely based on Bordet's and Gengou's results obtained by their method of testing for the presence of "fixateur." The results obtained by the use of that method are not above criticism. That the amount of fixateur present in normal serum compared with immune serum is small is true, but, as suggested by Savtschenko, the amount necessary to prepare the organism for phagocytosis may be small compared with that necessary for bacteriolysis. When microbes, *e.g.*, streptococci, injected into the peritoneal cavity, come in contact with the phagocytes, at first they are englobed by these, but soon some are observed to be free and to multiply rapidly, apparently having the power of repelling leucocytes. Bordet and Savtschenko interpret this to mean that the cocci have acquired during this brief period a new property which gives rise to a negative chemiotaxis of the leucocytes.

In the light of my experiments another view may be taken, viz., that the first organisms injected are phagocyted, because they have been sensitised by the immune substance present in the normal serum. This being small in amount is soon exhausted, and the few organisms which may have escaped its action are able to multiply, and either are indifferent to the leucocytes or exercise their repelling influence on them in the absence of the naturally present immune substance. In such a case the indifference displayed by the phagocytes to the cocci, or it may be the repulsive force of the cocci, is not a newly-acquired property, but is inherent in the cocci, and is only overcome by the presence of the immune substance which acts as intermediary between the micro-organism and the leucocytes.

Conclusions.

1. That, as has been shown by a number of workers, *e.g.*, Denys, Metchnikoff, Savtschenko, Levaditi and others, there is produced in the blood serum of animals actively immunised by bacterial injections a specific immune substance which has among its properties that of preparing the microbe for phagocytosis.
2. That this immune substance is thermostable, resisting a temperature of 60° C. for several hours.

3. That in normal serum there is present a substance having a similar action and which also resists a temperature of 60° C. for hours, and may persist in the serum of the horse for years.

4. That the experiments recorded in this paper tend to confirm the idea that the substances are identical, *i.e.*, that in normal serum there is present a small amount of the immune substance having the property of preparing the microbes for phagocytosis.

5. That cocci fully occupied by the substance from heated immune serum when passed through fresh normal serum do not remove the substance from normal serum, whereas fresh cocci remove a large part of it.

6. That the converse of the above is also true, viz., that cocci fully occupied by the substance from normal serum do not remove the substance from immune serum, whereas fresh cocci do.

7. That the thermostable substance in normal serum is no doubt identical with the "fixateur" or "substance sensibilisatrice" of the French school and with Wright and Douglas' "opsonin."

Seeing that the terms "fixateur" and "substance sensibilisatrice" which have been employed by Metchnikoff's school to include the property of preparing the microbes for phagocytosis are used to designate a number of other properties of immune serum, it may be convenient to adopt Wright and Douglas' term of "opsonin" for the particular property in question. The only danger attached to such a course is that one might be led to regard the "opsonin" as actually a different substance and not merely a property of immune serum.

I wish here to express my thanks to Drs. MacConkey and Petrie for the kind assistance they have given me in making a large number of the enumerations.

REFERENCES.

Bordet, J. (1895). "Les Leucocytes et les Propriétés Actives du Sérum chez les Vaccinés." 'Annales de l'Institut Pasteur,' vol. 9, p. 462.

Bordet, J. (1897). "Contribution à l'Étude de Sérum Antistreptococcique," 'Annales de l'Institut Pasteur,' vol. 11, 1897, p. 177.

Bulloch, W., and Atkin, E. E. (1905). "Experiments on the Nature of the Opsonic Action of the Blood Serum," 'Roy. Soc. Proc.,' vol. 74, p. 379.

Denys (1897). "Résultats obtenus par le Sérum Antistreptococcique," 'International Medical Congress at Moscow,' Section III, p. 82.

Denys and Leclef, I. (1895). "Sur le Mecanisme de l'Immunité chez le Lapin Vacciné contre le Streptocoque Pyogène. La Céllule" (1895), p. 177, vol. 11.

Hektoen, L., and Ruediger, G. F. (1905). "Studies in Phagocytosis," 'Journal of Infectious Diseases,' vol. 2, p. 128.

Levaditi, C. (1901). "Sur l'État de la Cytase dans le Plasma des Animaux normaux et des Organismes vaccinés contre le Vibrion Cholérique," 'Annales de l'Institut Pasteur,' vol. 15, 1901, p. 894.

Mennes, Fr. (1897). "Das Antipneumokokkenserum und der Mechanismus der Immunität des Kaninchens gegen den Pneumokokkus," 'Zeitschrift für Hygiene,' vol. 25, p. 413.

Neufeld, F., and Rimpau, W. (1904). "Über die Antikörper des Streptokokken- und Pneumokokken-Immunserums," 'Deutsche Medizinische Wochenschrift,' Jahrgang 30, p. 1458.

Savtschenko and Melkich (1901). "Étude sur l'Immunité dans le Fièvre recurrente," 'Annales de l'Institut Pasteur,' vol. 15, p. 498.

Savtschenko, J. G. (1902). "Du Rôle des Immunesines (Fixateurs) dans la Phagocytose," 'Annales de l'Institut Pasteur,' 1902, vol. 16, p. 106.

Wright, A. E., and Douglas, S. R. (1903). "An Experimental Investigation of the *Rôle* of the Blood Fluids in connection with Phagocytosis," 'Roy. Soc. Proc.,' vol. 72, p. 358.

The Phagocytosis of Red Blood-Cells.

By J. O. Wakelin Barratt, M.D., B.Sc. Lond., British Medical Association Research Student.

(From the Hygienisches Institut, Munich. Communicated by Sir Victor Horsley, F.R.S. Received July 14, 1905.)

It has been shown by Savtschenko,* that when an animal has received injections of red blood-cells, its serum (inactivated by heating) causes the appearance of phagocytosis *in vitro* when leucocytes and red blood-cells of the kind used for injection are added, and the whole maintained at the temperature of the body. This action is attributed by Savtschenko to the action on the red blood-cells of amboceptor (immunisine, fixateur) contained in the serum employed.

In order to obtain further information respecting the factors which determine phagocytosis *in vitro* of red blood-cells, a comparative examination of the action of sera of animals nearly related to, and widely separated from, those supplying the erythrocytes used for injection was undertaken. This investigation confirmed the above observation, that leucocytes placed in the inactivated serum of injected animals ingested red blood-cells of the kind used for injection. In the course of this investigation, however, it was found that phagocytosis could be brought about by the serum of the injected animals when the serum was free from amboceptor, for the red blood-cells injected, as the following experiment shows:—

Experiment 1.—Into the abdominal cavity of a dove, the red blood-cells from the following amounts of hen's blood were injected: on the 1st day, 6 c.c.; on the 6th day, 6 c.c.; on the 15th day, 9 c.c.; on the 38th day, 5 c.c. On the 45th day the animal was killed. Some leucocytes from a dove, together with erythrocytes from a hen, were added to a small quantity of the serum of the injected animal. After the lapse of an hour, at 37° C., about half the leucocytes present contained one or two, or rarely more, red blood-cells. 0·5 c.c. of the serum of the injected animal was added to 1 c.c. of a 2·5 per cent. suspension in 0·85 per cent. sodium chloride solution of hen's erythrocytes, and the volume of the mixture then made up to 2 c.c. by the further addition of 0·5 c.c. of the salt solution. At the end of 5 minutes agglutination was complete; at the end of 24 hours, at 37° C., no trace of hæmolysis was perceptible. Smaller amounts of serum were also employed, with negative results, as far as the production of hæmolysis is concerned.

---

* "Du Rôle des Immunesines (Fixateurs) dans la Phagocytose," 'Annales de l'Institut Pasteur,' 1902, vol. 16, p. 106.

It follows from this experiment, therefore, that the presence of amboceptor is not causally related to the development of phagocytosis, since phagocytosis can occur in the absence of amboceptor.

It was also found that, in most of the experiments made, agglutinin was present, and the enquiry presented itself whether agglutinin was essential to phagocytosis. It was found, however, that in some cases phagocytosis made its appearance in sera which possessed no agglutinating power for the red blood-cells employed. Among experiments illustrating this point are the following:—

Experiment 2.—Into the abdominal cavity of a rabbit, the red blood-cells obtained from the following amounts of calf's blood were injected: on the 1st day, 20 c.c.; on the 6th day, 20 c.c.; on the 17th day, 80 c.c. On the 24th day blood was taken from the animal and serum obtained. To a small amount of this serum, after inactivation by heating to 58° C. for 30 minutes, leucocytes from the rabbit and erythrocytes from the calf were added. At the end of half an hour at 37° C., well-marked phagocytosis had occurred, about one-third of the leucocytes present having taken up one to three red blood-cells. To test the agglutinating power of the serum, which before inactivation was strongly hæmolytic, the following tests were made: to four test-tubes, each of which contained 1 c.c. of a 2·5 per cent. suspension (in 0·85 per cent. NaCl solution), 0·5 c.c., 0·25 c.c., 0·1 c.c., and 0·05 c.c. of inactivated serum respectively were added, and the bulk of fluid in each test-tube made up to 2 c.c. At the end of 24 hours, at a temperature of 37°, no agglutination had occurred in any of the tubes.

Experiment 3.—Into the abdominal cavity of a guinea-pig the red blood-cells obtained from the following amounts of calf's blood were injected: on the 1st day, 3 c.c.; on the 3rd day, 10 c.c.; on the 14th day, 7 c.c.

On the 30th day blood was taken from the animal and serum obtained. To a small amount of this serum (which when active was strongly hæmolytic), after inactivation, leucocytes from the guinea-pig and erythrocytes from the calf were added. At the end of half an hour, at 37° C., one-quarter of the leucocytes present were found to contain one to three or more red blood-cells. On testing the agglutinating power of the serum, as in the preceding experiment, no agglutination was observed in any of the test-tubes, even at the end of 24 hours.

Experiment 4.—Into the abdominal cavity of a guinea-pig the red blood-cells obtained from the following amounts of goat's blood were injected: on the 1st day, 10 c.c.; on the 6th day, 12 c.c.; on the 31st day, 12 c.c.; on the 41st day, 15 c.c. On the 49th day blood was taken from the animal and serum obtained. To a small amount of this serum, after inactivation, leucocytes from the rabbit and erythrocytes from the goat were added. At the end of half an hour at 37° C., well-marked phagocytosis had occurred, about one-third of the leucocytes containing red blood-cells; at the end of an hour more than three-quarters of the leucocytes had taken up red cells, which were observed to be more or less pale. On testing the agglutinating power of the serum, as in Experiment 3, no agglutination was observed in any of the test-tubes, even at the end of 24 hours.

From these experiments it follows that the presence of agglutinin is not a necessary factor in the production of phagocytosis.

The former of the above conclusions is confirmed by the behaviour of normal rabbit serum. This has a marked hæmolytic action on the red blood-cells of the goat and guinea-pig. Nevertheless in the combination: *inactivated normal rabbit serum + erythrocytes of goat + leucocytes of rabbit,* and *inactivated normal rabbit serum + erythrocytes of guinea-pig + leucocytes of rabbit* phagocytosis *in vitro* does not occur, so that in normal serum the presence of amboceptor is insufficient to bring about phagocytosis. None of the normal sera employed possessed a powerful agglutinating action upon any of the red blood-cells employed, so that a similar demonstration of the inefficiency of agglutinin cannot be given.

Since the sera of animals into which red blood-cells have been injected is capable of bringing about phagocytosis when amboceptor and agglutinin are absent, it follows that this property of such sera is due to some special substance which is not amboceptor or agglutinin.

Respecting the nature of this special substance, the following further data are available:—

1. The substance in question is, as is well known, withdrawn from the sera by red blood-cells of the kind used for injection.

Experiment 5.—The serum of a rabbit, into whose peritoneal cavity red blood-cells derived from the hen had been injected was found, in the combination: *inactivated serum + erythrocytes of the hen + leucocytes of rabbit*, to bring about a very active phagocytosis of red blood-cells at 37° C. When 1 c.c. of the inactivated serum was added to the red blood-cells (previously washed with 0·85 per cent. sodium chloride solution in order to remove serum) of 0·25 c.c. of hen's blood, and the whole allowed to remain three hours at 0°, it was found that, after removal of the red blood-cells by centrifugalisation, the serum after the addition of erythrocytes and leucocytes as above, had lost its power of bringing about phagocytosis.

Experiment 6.—The serum of a rabbit, into whose peritoneal cavity red blood-cells derived from the calf had been injected, was found in the combination: *inactivated serum + erythrocytes of calf + leucocytes of rabbit*, to bring about phagocytosis at 37°. When the inactivated serum was treated with the red blood-cells of the calf, as in the preceding experiment, it was found subsequently to have lost the power of causing phagocytosis.

Experiments 7, 8, 9 were similarly carried out with guinea-pigs which received injections of the red blood-cells of the hen, calf, and rabbit respectively. In each case the inactivated serum employed, which was capable of bringing about very active phagocytosis *in vitro*, after treatment for three hours at a temperature of 15° C. with one-quarter of its volume of red blood-cells of the kind used for injection, was found to have lost its power of bringing about phagocytosis *in vitro*.

Thus the material, the existence of which in serum confers on the latter the property of exciting phagocytosis, combines with, or attaches itself to, the corresponding red blood-cells, and can therefore be withdrawn from the serum in the same way as amboceptor or agglutinin.

2. On the other hand, as the following experiments show, this material is not, in the same period of time, withdrawn from serum to the same extent by leucocytes.

Experiment 10.—To 0·3 c.c. of the serum employed in Experiment 6, leucocytes from the rabbit, occupying (after centrifugalisation) a volume of about 0·3 c.c., were added. After remaining at a temperature of 37° for three hours, the mixture was centrifugalised and to a portion of the supernatant liquid, fresh leucocytes from the rabbit and red blood-cells from the hen, were added. At the end of an hour at 37° C., phagocytosis was found to have taken place, about 20 per cent. of the leucocytes present having ingested one to three red blood-cells.

Experiment 11.—The above experiment was repeated with the serum employed in Experiment 7, about 0·2 c.c. leucocytes being added to 0·2 c.c.

serum. On testing the phagocytic power of the serum by adding to it, at the end of three hours, leucocytes from the rabbit and red blood-cells from the calf, it was found that at the end of one hour, at 37°, about 25 per cent. of the leucocytes had taken up one to four red blood-cells.

Experiments 12 *and* 13.—These were similarly carried out with the serum of guinea-pigs, which had received intraperitoneal injections of red blood-cells of the calf and rabbit respectively. After the sera had been treated for three hours at 37° with an equal bulk of leucocytes from the guinea-pig, it was found on testing their phagocytic power that, at the end of an hour, 20 per cent. and 75 per cent. respectively of the leucocytes had taken up red blood-cells.

The *rôle* of the leucocytes, therefore, appears to be passive so long as the substance in question remains free in the serum, and only when a combination with red blood-cells has occurred do the leucocytes proceed to ingest the latter. Moreover, in experiments upon phagocytosis leucocytes other than those of the animal employed for injection of red blood-cells, may often be used. Thus it was found that when rabbits had been injected with red blood-cells from the hen or guinea-pig, their sera induced phagocytosis of red blood-cells of the kind injected, when leucocytes from the guinea-pig, goat, or sheep, instead of the rabbit, were used for experiment. In the same way with serum obtained from a goat injected with red blood-cells of the sheep, phagocytosis of the latter *in vitro* could be obtained with leucocytes from the rabbit, guinea-pig, sheep, or dove; and such examples can be multiplied.

3. When red blood-cells which have remained for some time in a serum capable of bringing about phagocytosis *in vitro*, are very thoroughly washed in 0·85 per cent. sodium chloride solution and then added to leucocytes suspended in saline solution, rapid phagocytosis may be obtained though the fluid employed is free from serum. It is essential in experiments of this kind that the serum used does not agglutinate the red blood-cells, for when the latter are heaped together phagocytosis cannot be satisfactorily observed. With the sera employed for Experiments 8 and 9, no agglutination occurred when red blood-cells from the calf and rabbit respectively were allowed to remain in four times their bulk of serum for one hour at 37°. After washing in saline solution and adding guinea-pig leucocytes, it was found that, at the end of 30 minutes at 37° C., 20 to 25 per cent. of the leucocytes had each taken up one to four red blood-cells. Still more rapid phagocytosis was obtained with red blood-cells of the calf and goat which had been similarly sensibilised with sera obtained from rabbits which had previously been several times injected with these varieties of red blood-cells.

4. The special constituent of serum which possesses the property of inducing phagocytosis is destroyed by heating.

Experiment 14.—The sera of three rabbits which were capable when diluted with four parts of saline solution of exciting active phagocytosis of the red blood-cells of the hen, calf, and goat respectively, were heated in the dilution of 1 in 5 for 30 minutes to 100° C. A slightly milky fluid free from precipitate was thus in each case obtained which, on testing, was found to have lost its power of causing phagocytosis. The sera of three guinea-pigs, injected with red blood-cells of the hen, calf, and rabbit respectively, were similarly found to have lost their power of causing phagocytosis, after 30 minutes exposure to 100°.

Experiment 15.—The six sera employed in the preceding experiment were heated, undiluted, to 69° for 30 minutes. All remained clear with the exception of two of the guinea-pig sera, which became slightly opaque and distinctly viscid. With these two no phagocytosis was obtainable; with the remaining sera phagocytosis was obtainable, but was not so vigorous as with unheated serum.

It appears, therefore, that while a temperature of 100° rapidly destroys the phagocytic action of serum, a temperature of 69° is much less effective.

5. The above characters serve to define the nature of the special constituent of serum which confers upon it the property of bringing about phagocytosis of red blood-cells, and show that its *rôle* is to prepare these cells for consumption by leucocytes. It is therefore a member of the group of opsonines first described by Wright and Douglas* in respect of bacteria.

In conclusion, it may be mentioned that erythrocytic opsonines are present in relatively small amount in normal sera. Thus, with the inactivated sera of the sheep, goat, dove, and hen, phagocytosis of the red blood-cells of the calf, goat, sheep, rabbit, and guinea-pig may, by suitably adjusting the conditions of experiment, be readily obtained. In the following experiment this is illustrated: in A the red blood-cells are insufficiently sensitised, so that no phagocytosis takes place, as is also the case in D, where the serum is apparently completely deopsinated; in F, on the other hand, owing to the relatively small amount of red blood-cells employed, the serum employed still retains sufficient opsonin to cause the appearance of phagocytosis when fresh red blood-cells are added.

---

* "The *Rôle* of the Blood Fluids in connection with Phagocytosis," 'Roy. Soc. Proc., 1904, vol. 72, p. 357; *cp.* "On the Nature of the Opsonic Action of the Blood Serum." W. Bulloch and E. E. Atkin, 'Roy. Soc. Proc.,' 1905, vol. 74, p. 379.

Experiment 16.—The following mixtures—

	A.	B.	C.
Inactivated serum of normal dove...	0·1 c.c.	0·1 c.c.	0·1 c.c.
Red blood-cells of guinea-pig	0·025 c.c.	0·005 c.c.	0·0015 c.c.

were kept (with constant shaking) at 30° C. for $2\frac{1}{2}$ hours. To a loopful of A, B, and C respectively, a few leucocytes from the dove were added; at the end of one hour none of the leucocytes added to A contained red blood-cells, while 15 and 20 per cent. respectively of those added to B and C contained red blood-cells. A, B, and C were now centrifugalised, and to the cell free sera fresh red blood-cells of the guinea-pig and leucocytes of the dove added (D, E, F respectively); at the end of an hour, at 37° C., in D, none of the leucocytes contained red blood-cells, in E and F, 1 and 20 per cent. respectively of the leucocytes contained red blood-cells.

Report on the Anatomy of the Tsetse-fly (Glossina palpalis).

By E. A. MINCHIN, M.A., Professor of Zoology and Comparative Anatomy in University College, London, late Fellow of Merton College, Oxford.

(Communicated by Professor E. Ray Lankester, F.R.S. Received August 17, 1905.)

The following description is based upon dissections and preparations made in the laboratory of the Sleeping Sickness Commission at Entebbe since my arrival here at the beginning of April. I hope on my return to England to work up my material into a detailed memoir on the anatomy and histology. Time does not suffice for me to complete my work out here, but it seemed worth while, nevertheless, to bring forward as soon as possible a brief description of the general anatomy of the fly, and especially of its digestive tract, on account of its importance for the study of the evolution of the trypanosomes of Sleeping Sickness, and other tsetse-fly diseases, within the body of their invertebrate host.

In this paper I do not propose to attempt to deal with either the muscular system or the respiratory tracheal system. The former of these is so complex that much more time would be required for working it out than I could afford to spend, and it is, moreover, of little or no importance for the aim in view; while the tracheal system, or at least its finer branches, are so intimately connected with the fat-body, which here, as in other insects, fills up the body-cavity, that in the process of clearing up and laying bare the organs, the tracheæ are for the most part removed. Special muscles or tracheæ will be mentioned in places, but otherwise no account will be taken of these two systems.

The drawings illustrating this memoir are to be regarded as semi-diagrammatic, but all details in them have been traced from sketches made with the camera lucida from actual dissections, and therefore claim to be true to nature and accurate as regards scale and proportions. For help in the preparation of these drawings I am much indebted to my colleague, Mr. F. Tulloch, R.A.M.C., who also kindly cut some sections for me. Mr. Tulloch has also made some dissections of *Stomoxys*, comparison with which has thrown light on some points in *Glossina;* Mr. E. Degen, who came out with me, has also helped me in various ways.

Since I have no access out here to any literature or works of reference dealing with insect-anatomy, I am unable to make this account comparative, or to state how far *Glossina* differs from other Diptera as regards internal structure. I shall content myself, therefore, with describing the facts observed by me in a purely objective manner.

In the following description I shall employ the term *waist* for the narrow peduncle connecting the thorax and abdomen, and *neck* for the still narrower connection between head and thorax.

1. *The Nervous System of Glossina,* as of other Diptera, is concentrated into two masses, one situated in the head, the other in the thorax.

The brain (fig. 1) consists of the two large cerebral ganglia (*S. O. G.*) giving off laterally the still larger optic lobes (*Op. l.*), from which arise the optic nerves. The dissection of the brain and its nerves is rendered somewhat difficult by the large air-sacs, dilatations of the tracheal system, contained in the head. From the anterior side of the cerebral ganglia various nerves are given off: first, a median nerve of moderate size to the three ocelli (*oc. n.*), arising from the furrow between the two cerebral ganglia, and apparently swelling out into a small ganglion; secondly, a pair of nerves to the antennæ, arising about half-way down the front of the brain on each side; thirdly, a pair of small nerves which innervate the muscles of the pharynx, arising near the base of the brain; and lastly, a pair of nerves to the proboscis, which arise from the base of the brain, run forward ventrally to the pharynx, giving off nerves at this point to the muscles of the proboscis, and finally enter the bulb of the proboscis, to be distributed to the mouth-parts (fig. 5, *n. p.*).

From the posterior surface of the brain, near its base, the two stout connectives (fig. 1, *Cn.*) arise, and pass down on each side of the greatly narrowed œsophagus, after which they unite almost immediately to form a single broad band of nerve-tissue, which runs back through the neck to join the thoracic ganglion-complex. From this connective band, as it may be termed, there arises, immediately after it enters the thorax, a slender pair of nerves, which form a delicate plexus with the first pair of prothoracic nerves arising a short way behind them (fig. 1, *Cn. n.*).

The connective band often appears distinctly double at its junction posteriorly with the thoracic ganglion-mass, which lies immediately ventral to the stomach, the anterior end of the former being a short distance behind that of the latter. It is a mass of considerable thickness in the dorsoventral direction, and appears more or less pear-shaped in a dorsal view, but seen from the ventral side its anterior end appears truncated. When stained, cleared, and mounted in Canada balsam, it is seen distinctly to be composed of three pairs of large ganglia united together, corresponding to the three segments of the thorax, behind which a small mass of ganglion-cells,

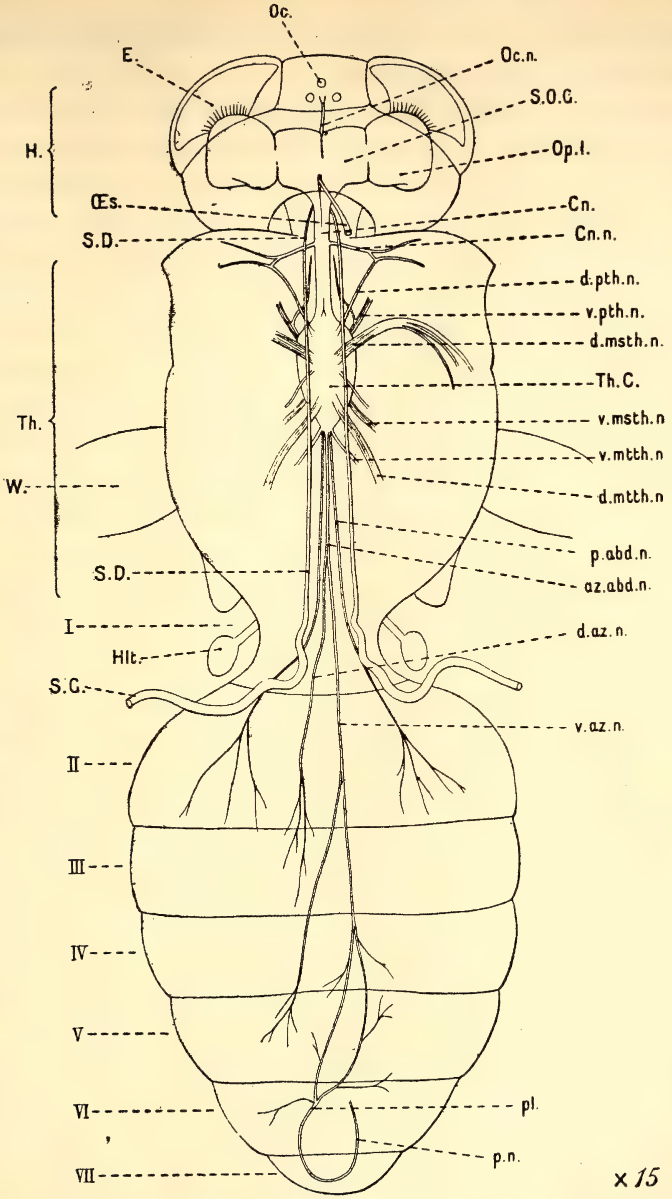


FIG. 1.—General Sketch of the Nervous System, dorsal view. The top of the head is pushed forward and downward as far as possible, to show the brain. A portion of the œsophagus and the salivary ducts are also represented, the salivary gland being supposed to be turned outwards from the abdomen and cut off near the origin of the ducts.

All lettering relating to the nervous system has been placed on the right of the figure, that referring to other parts on the left. *H.*, head; *Th.*, thorax; I—VII, the segments of the abdomen; *Oc.*, ocelli; *E.*, eyes; *Œs.*, œsophagus (cut off); *S. D.*, *S. D.*, salivary duct; *W.*, origin of wings; *Hlt.*, halter; *S. G.*, salivary gland; *Oc. n.*, ocellar nerve: *S. O. G.*, right cerebral ganglion; *Op. l.*, right optic lobe; *Cn.*, connectives; *Cn. n.*, nerve from the connective forming a plexus with: *d. pth. n.*, dorsal prothoracic nerve; *v. pth. n.*, ventral prothoracic nerve; *d. msth. n.*, *v. msth. n.*, dorsal and ventral mesothoracic nerves; *Th. c.*, thoracic ganglion complex; *d. mtth. n.*, *v. mtth. n.*, dorsal and ventral metathoracic nerves; *p. abd. n.*, paired abdominal nerve; *az. abd. n.*, azygos abdominal nerve; *d. az. n.*, dorsal branch of the azygos nerve; *v. az. n.*, ventral branch of the azygos nerve (genital nerve); *pl.*, plexus formed by the genital nerve; *p. n.*, nerve to penis.

representing the abdominal nervous system, forms the posterior-pointed termination of the thoracic complex.

From each of the thoracic ganglionic centres arise two nerves, one dorsal and one ventral, so that altogether six pairs arise from the body of the thoracic complex, which are distributed to their proper regions of the thorax. From the posterior end of the thoracic complex arise three nerves, one median unpaired, and two lateral paired, which pass backwards into the abdomen.

The greater part of the thorax of the fly is a mass of muscle, and as the muscles have to be removed in order to display the other organs in the thorax, the terminations and finer branches of the nerves are torn away from them. Hence it is impossible to describe accurately the destinations of these nerves without a detailed study of the musculature, which, as already stated, I have not made. It would appear, however, that the three ventral pairs of thoracic nerves innervate the legs and their muscles.

The dorsal prothoracic nerves (fig. 1, *d. pth. n.*) are very slender, and, as already stated, form an anastomosis with the nerves from the connectives. The ventral prothoracic nerves (*v. pth. n.*) are of moderate size.

The dorsal mesothoracic nerves (*d. msth. n.*) are very large, being in fact the stoutest nerves in the body. They run slantingly forward, then curve round till they run in a backward direction, and appear to be distributed to the wing-muscles. A small nerve arises from the ganglion close behind the origin of the dorsal mesothoracic nerves, and runs backwards in a dorsal direction. It is drawn in fig. 1, but not lettered, and is probably to be regarded as a branch of the dorsal mesothoracic nerve. The ventral mesothoracic nerves (*v. msth. n.*) are also of fairly large size.

The dorsal metathoracic nerves (*d. mtth. n.*) are large, the ventral ones (*v. mtth. n.*) of moderate size.

The three abdominal nerves run at first straight backwards, and almost parallel to each other, to the waist. Before reaching it the median nerve (*az. abd. n.*) has divided into a smaller dorsal and a larger ventral branch. After passing through the waist the two lateral nerves (*p. abd. n.*) diverge outwards to the sides of the abdomen and break up into numerous branches.

The dorsal branch of the median nerve is distributed to organs situated dorsally in the abdomen. The ventral branch of the median nerve is the nerve of the generative organs. In the male I have found that its branches unite to form a plexus (fig. 1, *pl.*), apparently containing a small ganglion, which gives off nerves in various directions, and from which a fairly stout nerve (*p. n.*) arises and follows the ductus ejaculatorious in its tortuous course, till it finally enters with it the penis, the muscles of which it innervates. In the female a similar plexus appears to be formed, but owing to the dense tangle formed by the fat-body, uterine glands, and Malpighian tubules, I have not succeeded in dissecting out its finer details.

2. *The Digestive Tract.*—Since the proboscis, buccal cavity, and pharynx have been thoroughly described in Austen's monograph by Hansen, whose account I can but confirm, I commence my description with the œsophagus. This portion of the alimentary canal (figs. 1 and 2, *Œs.*) runs first of all in an upward direction from the pharynx (*Ph.*), then bends sharply round and passes backwards through the brain. The first portion of the œsophagus is dilated, but slightly compressed, appearing of greater calibre in a dorsal than in a lateral view. After bending round, it narrows rapidly, and the portion which passes through the brain is of extreme tenuity, scarcely, if at all, of greater calibre than the salivary ducts. Behind the brain the œsophagus widens, at first very gradually, then, after entering the thorax, more rapidly, till it joins the stomach, into which it opens ventrally, breaking through the floor slightly in front of the point at which the thoracic intestine arises dorsally. From the point at which the œsophagus opens into the stomach, the duct of the sucking stomach arises.

The stomach, which marks the commencement of the mesenteron, has a peculiar and very characteristic form (fig. 2, *St.*). Seen from the dorsal aspect, it appears roughly oblong in form, with a bevelled anterior edge, and the upper surface more or less saddle-shaped, *i.e.*, convex in the transverse section, concave in the longitudinal direction. Seen from below, its lateral edges appear wrapped round the œsophagus and the duct of the sucking stomach, on which it rides, as it were. From the dorsal side of the stomach, about the middle of its length, arises the intestine, the first part of which (*Th. I.*) runs backwards through the thorax as a straight tube of even calibre, until it passes the waist. As soon as it enters the abdomen the intestine swells and becomes the strictly digestive portion of the alimentary canal.

The abdominal intestine is of great length, but until it reaches the proctodæum it cannot be divided into regions. It forms a number of complicated coils in the abdomen, and for purposes of description a number of limbs may be distinguished, each limb separated from the one next following by a more or less sharp bend (figs. 2 and 3).

The first limb (1) runs backwards along the abdomen in a dorsal situation, curving first slightly to the right, then more strongly to the left, until it reaches the fifth segment, where a sharp bend takes place forwards in a ventral direction. The second limb (2) curves round from left to right,

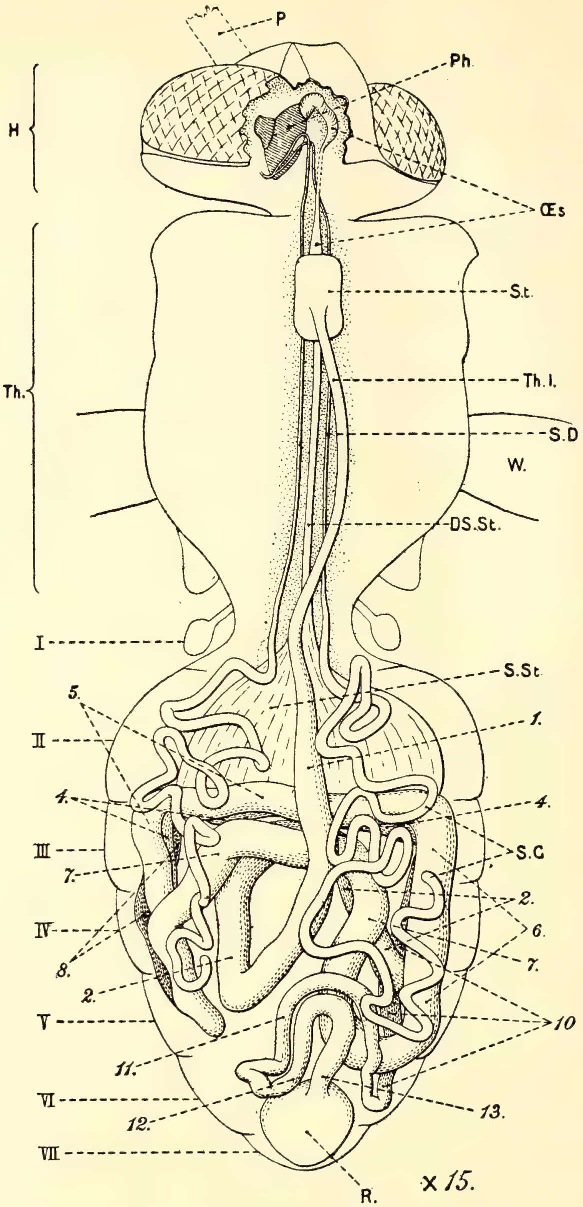


FIG. 2.—General View of the Digestive Tract, as seen in dorsal view without disturbing its parts. The heart and overlying tracheæ and fat-body are removed in the abdomen, also the muscles in the thorax, and the brain and other parts of the nervous system are omitted from the drawing. The head is turned round to the left, in order to show the pharynx, etc., in three-quarter side view.

Ph., pharynx ; *Œs.*, œsophagus (the portion which passes through the brain being represented with a dotted outline) ; *St.*, stomach ; *Th. I.*, thoracic intestine, pulled over to the right, in order to show the duct of the sucking stomach lying beneath it ; *S. D.*, salivary duct ; *DS. St.*, duct of : *S. St.*, the sucking stomach ; *S. G.*, salivary gland (that on the right is drawn from a specimen in which the gland was more developed than in the case of that drawn on the left) ; 1—13, limbs of the abdominal intestine (see fig. 3) ; *R.*, rectum. Other letters as in the preceding figure.

running first anteriorly, then transversely, and lastly in a posterior direction. It is more ventral in situation, being placed below some of the succeeding coils. The third limb (3) is short, and runs straight forward on the right side from the fifth to the third segment. Not visible in fig. 2, its position is indicated in fig. 3. The fourth limb (4) turns at right angles, and runs transversely across the body in the third segment, passing back a short distance into the fourth. The fifth limb (5) turns sharply back on the fourth and runs dorsally to it across the body again in the third segment. The sixth limb (6) turns back at a right angle and runs back on the right side of the body from the third to the sixth segment. The seventh limb (7) turns sharply forwards, then curves round in a roughly semi-circular course in the third segment, and finally runs backwards on the left side as far as the fifth segment. The fifth, sixth, and seventh limbs form together a well-marked loop, lying superficially, which is generally the most dilated portion of the intestine. The eighth limb (8) bends sharply forwards and downwards, and runs deep on the left side from the fifth to the third segment. The ninth limb (9) bends at right angles and runs at first transversely in the third segment, then curves back into the fourth, then forwards again into the third segment. The tenth limb (10) runs backwards along the right side of the body from the third to the sixth segment, and in the fifth segment gives off

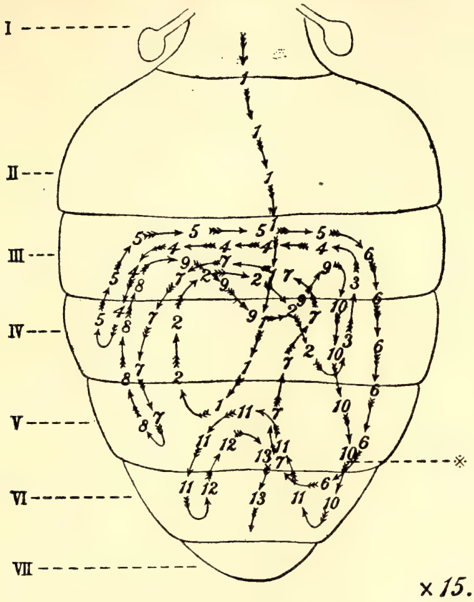


FIG. 3.—Diagram to show the various limbs (1—13) of the abdominal intestine, and their arrangement in the abdomen. The asterisk * denotes the point at which the Malpighian tubules arise in the tenth limb.

the Malpighian tubules (*, fig. 3), so that from this point the gut must be regarded as proctodæum. The eleventh limb (11) runs from right to left in a semicircular curve occupying the fifth and sixth segments. From the origin of the Malpighian tubules to the end of the eleventh limb the gut is of small calibre, and may be called the ileum. The succeeding portion is thicker, and may be called the colon. It lies in the fifth and sixth segments, and forms the twelfth and thirteenth limbs (12, 13), both short and sharply bent one on the other. The ileum and colon lie dorsally in the body, and the colon passes into the capacious rectum (*R.*), which has four rectal glands (fig. 5, *r. gl.*) each supplied by a bunch of small tracheæ.

The appendages of the digestive tract are the salivary glands, the sucking stomach, and the Malpighian tubules.

The salivary glands (fig. 2, *S. G.*) commence, starting from their distal ends, as two long tubes, much coiled, and occupying a very superficial dorsal position in the abdomen on each side of the heart, above the alary muscles. Very transparent in the fresh condition, the salivary glands become glistening white in colour when put in alcohol. Only tracheæ and fat-body come between them and the dorsal body-wall. The coils of the tubes extend back as far as the fourth or fifth abdominal segment, but the distal extremity of the gland may lie further forward than this. With many twists and turns the tubes run forward to the waist and then pass into the thorax, at the same time diminishing rapidly in calibre, straightening out their coils, and descending to the ventral side of the body. From this point the salivary gland becomes the salivary duct (figs. 1 and 2, *S. D.*). The two ducts run a parallel course through the thorax, on a level with the duct of the sucking stomach, and on each side of it, passing under the stomach and above the thoracic ganglion (figs. 1 and 2). When they reach the neck, the salivary ducts become so extremely attenuated that their course through the head is very difficult to follow. As they enter the neck the ducts curve over towards each other, and pass under the connective nerve-band, thus parting company from the œsophagus, which passes above the connective. The ducts pass under the brain and then under the pharynx.

If the head of a fly be examined from below, there will be found, immediately behind the bulb of the proboscis, an area covered by soft flexible integument, which recalls the soft skin at the base of a parrot's beak, and has a similar function, that is to say, to allow free play for the movements of the proboscis. When the proboscis is bent down, in the attitude it assumes when the fly is drawing blood, the soft skin forms a fold over the bulb, and when the proboscis points forward, in the attitude of repose, enclosed in the sheath formed by the two palpi, then the soft integument is stretched. If this flexible skin be removed, a cavity is exposed lying below the pharynx (fig. 4, *Ph.*), across which run the nerves

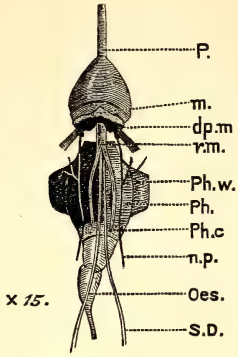


Fig. 4.—Dissection of Pharynx, Proboscis, Salivary Ducts, etc., ventral view.

P., proboscis ; *m.*, soft integument cut away behind the bulb of the proboscis ; *dp m.*, depressor muscle of the proboscis ; *r. m.*, retractor muscles ; *Ph.*, pharynx ; *Ph. w.*, chitinous wings of the pharynx ; *Ph. c.*, membranous continuation of the ventral wall of the pharynx, to which the retractor muscles are attached ; *n. p.*, nerve to the proboscis ; *Œs.*, œsophagus ; *S. D.*, salivary duct.

to the proboscis (*n. p.*), a pair of retractor muscles uniting anteriorly (*r. m.*) and the two delicate salivary ducts (*S. D.*). The last-named remain perfectly distinct and separated from one another until they pass dorsally to the median muscle formed by the two united refractors. A stained preparation, which I have cleared and mounted in Canada balsam, shows the two ducts uniting into a single duct above this muscle. In *Stomoxys*, according to Hansen's description, the two salivary ducts unite into a median duct much further back than I have found to be the case in *Glossina*. Hansen, it may be noted in passing, speaks always of the thoracic salivary gland,* but in *Glossina* these glands are not thoracic, and in *Stomoxys* they are partly abdominal. The immensely powerful muscles of flight, filling up the thorax, are probably the cause of the glands being shifted back into the abdomen. To follow the further course of the salivary duct after it enters the proboscis, sections would be required, which I have not made, since Hansen has already described the duct as opening on the hypopharynx, as in all other insects.

The sucking stomach is morphologically a ventral diverticulum of the distal end of the œsophagus, which is placed in the two anterior segments of the abdomen, its connection with the œsophagus being drawn out into a long slender duct traversing the thorax. The sucking stomach in the ordinary condition of the fly is filled with gas, but shortly after feeding it is found filled with blood.

---

* Austen also states ("Monograph," p. 35) that "the salivary gland [of Diptera] . . . is always situated in the *thorax*." (The italics are Austen's.)

The duct of the sucking stomach arises, as already stated above, from the œsophagus, at the point at which the latter communicates with the stomach, in such a way as to appear as a direct continuation of the œsophagus, the opening into the stomach having rather the appearance of a dorsally-directed diverticulum. At the point where the communication with the stomach occurs, the sides of the stomach are folded down ventrally so as to wrap completely round the duct, meeting below it, and forming a complicated system of cavities into which the fat-body intrudes.

When the duct passes the waist, it expands rapidly to become the capacious sucking stomach (figs. 2 and 5, *S. St.*), which has delicate walls, provided with a layer of unstriped muscles disposed irregularly.

The Malpighian tubules (*M. t.*, *M. t.*, fig. 5) arise by a pair of main stems given off from opposite sides of the 10th limb of the abdominal intestine. Each of these stems very soon divides into two again. In *Glossina* these tubules are excessively long, and so entangled with the fat-body and other organs that it is impossible to unravel them for their whole length, but since they are never observed to branch again, after their origin from the two main stems, it may be inferred that, as in other Diptera, there are in all four Malpighian tubules, disposed in this case in two couples, each couple coming off from a common stem. When the dorsal integument of the abdomen is removed, it can generally be observed without difficulty that two of the Malpighian tubules have thickened terminations, which lie close alongside the heart in the pericardial sinus right and left. In some specimens of *Glossina* these two tubules are not conspicuously thickened, but their position is constant. In no case do they exceed the salivary glands in thickness. It is evident that these two tubules must be of physiological importance for purifying the blood in the pericardial sinus. Mr. Tulloch has found in *Stomoxys* the same two pericardial Malpighian tubules, thickened to such an extent as to greatly exceed in calibre the salivary glands. Mr. Tulloch also found, and I was able to confirm his observation, that the two pericardial tubules of *Stomoxys* were a couple, arising both from one of the two stems on one side of the gut. The Malpighian tubules being much shorter in *Stomoxys* than in *Glossina*, it was possible to dissect out the two pericardial tubules of the former as far as their common origin from the gut, at which point they were detached, stained, and mounted in Canada balsam, thus putting this somewhat unexpected result beyond all doubt. Whether the two pericardial Malpighian tubules of *Glossina* are also, like those of *Stomoxys*, a couple with a common origin, cannot be stated with complete certainty, but it seems at least highly probable. The morphological significance of this fact is, perhaps, that the two common stems of the Malpighian tubules are not to be considered as arising right and left from the gut, but as dorsal and ventral in origin. I have not succeeded in finding the distal extremities of the two remaining tubules, but they appear to pass down towards the ventral side of the abdomen and to be entangled with the genital organs, the dissection of which they help to render difficult.

3. *The Genital Organs* lie in both sexes close to the ventral side of the body in the hinder segments of the abdomen.

The male organs (fig. 5) consist of two pairs of tubes, greatly convoluted for a whole or a part of their course, which open all together into an unpaired tube, the ductus ejaculatorius, which in its turn passes to the external organs of generation and opens on the penis.

Commencing with the paired portions of the male apparatus, it is observed that the two tubes on either side differ markedly from one another. One pair, placed most posteriorly, is tightly wound and has the coiled portion pigmented. The other pair, more anterior, forms a looser coil and is without any pigment. I identify the former as the testis, the latter as the vesiculæ seminales.

Each testis commences with a delicate white filament (*t. f.*), embedded in the fat-body and difficult to trace. I have not succeeded in finding where the free end of the filament is attached; in dissections it appears to be loose. The filament passes on into the tightly coiled pigmented tube, which forms a conspicuous, compact, brown body, the testis (*T.*). In one dissection I succeeded accidentally in uncoiling the testis by pulling inadvertently on the filament when trying to remove the fat-body. It was then seen that each testis is a whitish coiled tube enveloped in a pigmented brown coat, which crumbles easily into a brown powder. In specimens that have been long in alcohol also the pigmented coat often sticks to the surrounding fat-body and comes away from the testis. The proximal part of the testicular tube is dilated and forms the testis proper; the distal portion is of smaller calibre and more tightly coiled, forming an epididymis, from which the tube is continued as the vas deferens (*V. d.*). The latter is a white, straight, or but slightly sinuous tube. The brown pigment of the testis is continued a very short way down the vas deferens, and ends abruptly.

Each vesicula seminalis (*V. s.*) is a white tube, commencing with a blind end. A short distance from the commencement the tube is slightly thickened for a short distance. There is nothing to bind the coils together, nor any pigment, as in the testis. Distally the tube straightens out to open into the unpaired duct of the generative system.

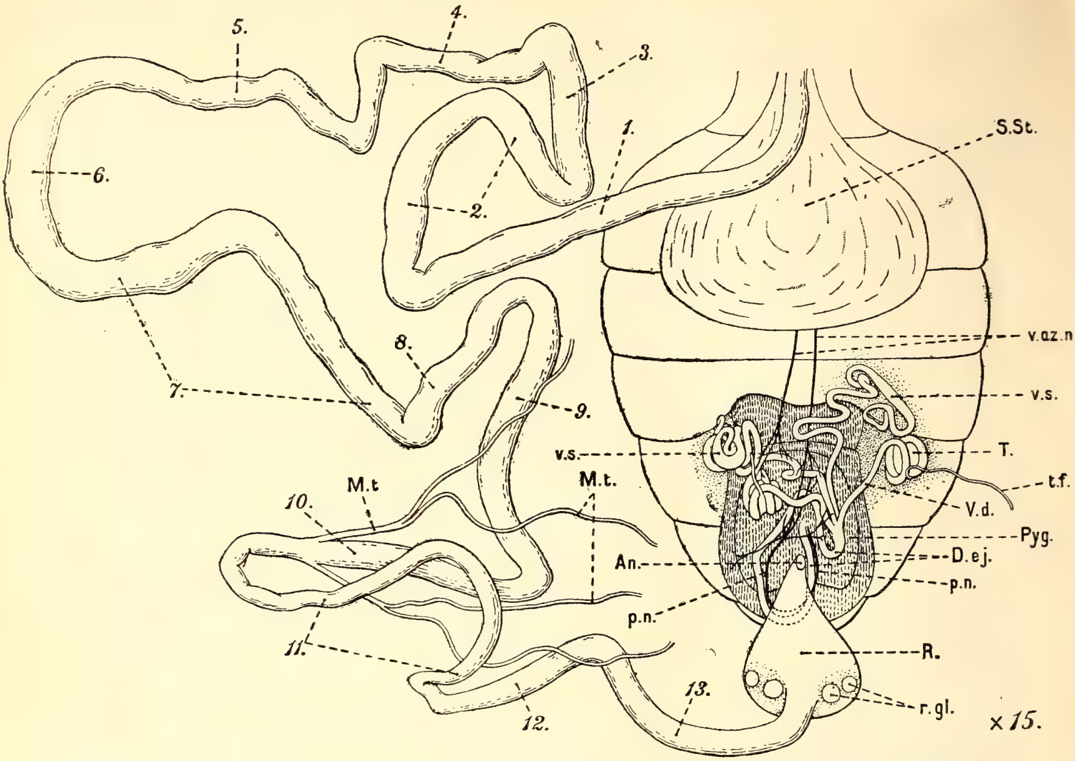


FIG. 5.—Dissection of the Abdomen, showing the Abdominal Intestine unravelled and turned over to the left side, and the Male Genitalia *in situ* in the Abdomen.

S. St., sucking stomach; 1—13, the limbs of the intestine, as indicated in the two previous figures; *M. t.*, *M. t.*, Malpighian tubules; *R.*, rectum; *r. gl.*, rectal glands; *An.*, anus; *T.*, testis; *t. f.*, testicular filament; *V. d.*, vas deferens; *v. s.*, *v. s.*, vesiculæ seminales, that on the left in its natural coil, that on the right unravelled; *D. ej.*, ductus ejaculatorius; *Pyg.*, hypopygium; *v. az. n.*, branches of the ventral azygos nerve (genital nerve); *p. n.*, nerve to penis, following the ductus ejaculatorius.

The ductus ejaculatorius (*D. ej.*) has at its commencement a slight dilatation, into which open the four tubes just described. From this point the ductus runs a very short way backwards, then curves sharply forwards, but soon turns back again, passes across to the left side ~~side~~ of the body, and forms a loop round the rectum, coming forward on the right to pass into the penis.

The various parts of the male generative organs are innervated, as already described, by a nerve plexus formed from the azygos abdominal nerve. There appears to be a small ganglionic swelling on the ductus ejaculatorius, whence arises a nerve (*p. n.*) which follows the ductus in its course to the penis.

The external organs of generation are concealed beneath the hypopygium. The penis is an organ of complicated structure and mechanism, with an armature of hooks, spines, hairs, and semaphore-like erectile flaps, which would require so many figures to make their arrangement and relations clear, that I refrain at present from attempting any description of them.

The female genital organs differ considerably in appearance according as they are in the gravid or non-gravid condition. In the course of my dissections I have only found one female in the latter state. In the later periods of gestation the condition of the female is obvious externally, but females which do not appear to be gravid are found on dissection to have a small larva in the uterus.

The female organs (fig. 6) consist, like the male, of paired and unpaired portions. The former comprise the ovaries, the receptacula seminis and their ducts, and the uterine glands; the latter are the oviduct, uterus, and

Fig. 6.

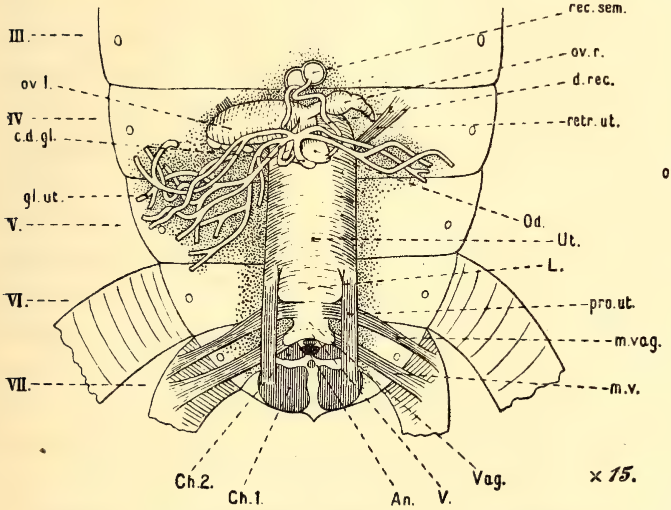


Fig. 7.

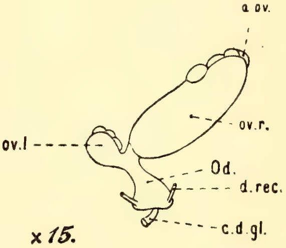


Fig. 6.—The Hinder Segments of the Abdomen with the Female Genital Organs *in situ*, dorsal view.

rec. sem., receptacula seminis; *ov.*, *r.*, *ov. l.*, right and left ovarioles; *d. rec.*, duct of the right receptaculum seminis; *gl. ut.*, uterine glands (the greater number of these have been removed); *c. d. gl.*, their common duct; *retr. ut.*, retractor muscle of the uterus; *Od.*, oviduct; *Ut.*, uterus; *L.*, hinder extremity of the larva, causing a bulge in the uterus; *pro. ut.*, protractor uteri, attached to the chitinous plate (*Ch.* 1); *m. vag.*, muscle (dilator vaginæ?) passing from the vagina to the tergum of the seventh abdominal segment; *m. v.*, muscle passing from the paired chitinous plate (*Ch.* 2) on each side of the vulva to the seventh tergum; *Vag.*, vagina; *V.*, vulva, the anterior margin of which is shown by a dotted line; *An.*, anus; *Ch.* 1, *Ch.* 2, paired chitinous plates.

Fig. 7.—The Ovarioles and Oviduct of a Non-gravid Female.

a. ov., apex of right ovariole; other letters as in the preceding figure. The very large ovum in the right ovariole has pushed the oviduct over towards the left side of the body.

vagina. The female system of organs is considerably modified from the condition usually found in insects, in relation to the fly's peculiar method of reproduction.

The ovaries are reduced to a single pair of ovarian tubes or ovarioles, one on each side of the body (figs. 6 and 7, *ov. r.*, *ov. l.*). Each ovariole shows only a small number of egg-chambers, not more than four or five. The lowest chamber is very much larger than any of the others, and contains a large ovum. When this ovum is comparatively small, the other egg-chambers are in a line with it (fig. 6, *ov. r.*), but as the ovum grows larger it grows past the other egg-chambers, so that they appear attached to the side of the ovum (fig. 6, *ov. l.*, fig. 7., *ov. l.*, *ov. r.*).

The two ovarioles are always asymmetrical, owing to the fact that the ova in the lowest egg-chambers reach full growth on each side alternately, so that if there is a large ovum on the left, there will be a smaller one on the right, and *vice versâ.* The largest ovum I have seen was from a non-gravid female (fig. 7, *ov. r.*), and was probably nearly, if not quite full-sized.

The two ovarioles open into the short, broad oviduct (figs. 6 and 7, *od.*), which widens out at its lower end to open into the uterus slightly behind the proximal end of the latter.

At its distal-expanded end the oviduct receives right and left the two ducts (*d. rec.*) of the receptacula seminis. The latter (*rec. sem.*) are small spherical bodies of a bright orange-yellow colour, surrounded by a whitish, transparent envelope. Examination of the receptacula stained and mounted in Canada balsam shows that the clear envelope is an epithelium of large cells, surrounding a thick chitinous membrane which gives these organs their peculiar colour, and which is too opaque for the contents to be seen except in sections, by which method the receptacula are seen to be filled with spermatozoa. The two receptacula are firmly attached to one another. From each comes off the slender white duct, slightly convoluted. The ducts are perfectly distinct from one another, and open, as described above, into the lower end of the oviduct.

Immediately below the opening of the oviduct into the uterus, a small tube debouches into the latter by a median dorsal aperture. This is the common duct of the uterine glands (figs. 6 and 7, *c. d. gl.*). After a short course it branches right and left into tubes, which branch again repeatedly, forming a great number of glandular tubes, which differ markedly in the gravid and the non-gravid condition. In the latter state the gland-tubes are relatively few and very slender. In the gravid condition, on the other hand, the tubes are very numerous, forming a tightly packed mass filling up the posterior end of the abdomen, and requiring to be pulled away to show the other parts of the generative system; further, the individual tubes are much thicker, and when stained and mounted, they take up the stain very deeply and appear very opaque. There can be no doubt that these glands serve for the nourishment of the larva in the uterus.

The uterus (*Ut.*) is a large thimble-shaped organ attached to the body-wall by a number of muscles. Two retractors (*retr. ut.*) run forwards from the proximal end. There are two pairs of protractors, one dorsal, the other ventral; the former (*pro. ut.*) start from the sides of the uterus and pass backwards to a pair of chitinous plates (*Ch.* 1) at the posterior end of the body. The wall of the uterus is beset by a very large number of small tracheal tubes (not shown in the figure), and is thick in the non-gravid condition, but becomes thinner when stretched by the growth of the contained larva. In all gravid uteri that I have seen, the two papillæ at the hinder end of the larva cause a bulge in the lower end of the uterus (fig. 6, *L.*). When the larva reaches a certain size, the rings of its segments become plainly visible through the wall of the uterus; they could not be seen in the uterus drawn in fig. 6, but in another, slightly larger, they could be seen distinctly.

The vagina (fig. 6, *Vag.*) is a broad tube, considerably longer in the non-gravid than in the gravid condition, with a pair of dilator muscles (*m. vag.*), which are attached right and left just below its junction with the uterus, and pass outwards to be attached to the anterior margin of the tergum of the seventh abdominal segment. The vagina widens out slightly as it approaches the vulva (*V.*), which is a crescentic, transversely elongated aperture, separated from the anus by a small chitinous plate (*Ch.* 2), one of a pair from which two muscles (*m. v.*) arise and pass outwards to be attached to the seventh tergum, a little way behind the attachment of the vaginal muscles already mentioned. These muscles probably act as dilators of the vaginal aperture, and the five pairs of muscles described in the preceding lines are to be regarded as constituting the mechanism of parturition.

4. *The Vascular System* consists of the heart, in the abdomen, and its continuation, the thoracic aorta, in the thorax.

The heart occupies the five anterior segments of the abdomen, and is situated dorsally immediately below the plates of the terga. It is so imbedded in the fat-body and pericardial tissue that not much can be made out of its structure by dissection alone, and examination of it mounted as a preparation for the microscope is necessary. It can then be seen to have five chambers, each with a pair of ostia and a pair of alary muscles, corresponding to the segments in which it lies. The alary muscles pass out at right angles to the axis of the heart, and can be traced through the fat-body to their attachments at the external lateral margins of the tergal plates.

The hindermost chamber of the heart appears to end blindly posteriorly. A little way in front of the hinder end are attached the two large alary muscles, the largest of the whole series; not far in front of these again are the two ostia, on the sides of the widest part of the chamber. In front of the ostia the lumen of the heart narrows rapidly, and to the narrowed portion is attached the next pair of alary muscles, lying in the hinder part of segment IV. This arrangement is continued in segments II, III, and IV, the dilated portion of the chamber, with the ostia, occupying the middle of the segment, while the alary muscles, attached to the constrictions between the chambers, lie in the posterior regions of the segments. The alary muscles of these three segments are of moderate size. In segment II the heart receives a pair of tracheal tubes, right and left, which come to it opposite the ostia, and fork at once into branches running forwards and backwards. The alary muscles corresponding to the first abdominal segment are very small and difficult to make out, and the region of the heart to which they are attached does not show the slightest diminution or constriction of its lumen, as is the case in all the chambers posterior to it. In front of the first pair of alary muscles, at the usual interval, are the two ostia, quite similar to those of the other chambers. In front of the first pair of ostia the lumen of the heart narrows to form a thin-walled vessel, which passes through the waist to become the artery which I have termed above the thoracic aorta. This last runs along the thoracic intestine on its dorsal side, and is continued over the stomach, remaining apparently quite independent of the digestive tract, and only loosely attached to it, until it reaches the œsophagus. Here it is firmly attached and becomes considerably dilated. A short distance in front of the stomach a conspicuous cushion-like mass of large cells lies over the aorta. At first I took this structure for a ganglion, but it appears to be a sort of lymphatic gland, judging from its appearance in sections. The thoracic aorta is apparently continued through the neck into the head, but I have not been able to follow its course further than the thorax.

The microscopic examination of the heart shows further that its floor is composed chiefly of fusiform cells resembling unstriped muscle-fibres, while its sides are made up of gigantic cells with nuclei of corresponding proportions. These cells are arranged with perfect regularity, and in a manner exactly similar on the two sides of the heart. Each ostium is formed by two cells, which are of small size when compared with the huge cells building up the wall of the heart, but are very large when compared with the cells of the surrounding tissues. Two of the giant cells intervene on each side between the hinder end of the heart and the fifth pair of alary muscles; two more between these muscles and the ostia next in front of them; and so on with unfailing regularity all the length of the heart, each ostium being separated from the alary muscles next in front or behind by just two giant cells. In front of the first pair of ostia are found two cells of the usual size on each side, then a pair of slightly smaller cells, which pass on into the walls of the thoracic aorta. Thus the entire wall of the heart is built up of 23 pairs of giant cells, not counting the ten couples of smaller cells which compose the five pairs of ostia; to wit, four pairs to each of the five chambers. two additional pairs behind the fifth pair of alary muscles, and one pair anteriorly, making the transition to the thoracic aorta. In view of the fact that the thoracic vessel is itself to be considered as a modified anterior portion of the heart, it is interesting to find that its delicate wall contains very large, flattened nuclei, arranged in pairs, right and left.

The alary muscles consist of delicate fibrils, arranged in an irregular fan-like manner, uniting into a stout muscle-fibre which is distinctly striated.

ENTEBBE, UGANDA,
July, 1905.

the ostia next in front of them; and so on with unfailing regularity all the length of the heart, each ostium being separated from the alary muscles next in front or behind by just two giant cells. In front of the first pair of ostia are found two cells of the usual size on each side, then a pair of slightly smaller cells, which pass on into the walls of the thoracic aorta. Thus the entire wall of the heart is built up of 23 pairs of giant cells, not counting the ten couples of smaller cells which compose the five pairs of ostia; to wit, four pairs to each of the five chambers, two additional pairs behind the fifth pair of alary muscles, and one pair anteriorly, making the transition to the thoracic aorta. In view of the fact that the thoracic vessel is itself to be considered as a modified anterior portion of the heart, it is interesting to find that its delicate wall contains very large, flattened nuclei, arranged in pairs, right and left.

The alary muscles consist of delicate fibrils, arranged in an irregular fan-like manner, uniting into a stout muscle-fibre which is distinctly striated.

ENTEBBE, UGANDA,
July, 1905.

The Dual Force of the Dividing Cell. Part I: The Achromatic Spindle Figure Illustrated by Magnetic Chains of Force.

By Marcus Hartog, M.A., D.Sc., F.R.U.I.

(From the Biological Laboratory of Queen's College, Cork.)

(Communicated by Sir W. Thiselton Dyer, F.R.S. Received December 1, 1904,—Read January 19, 1905.)

[Plates 9—11.]

I.

Our first description of the polarised figure shown by the cytoplasm of the cell preparing for fission is due to Hermann Fol, who wrote in 1873: "Auf beide Seiten dieser Kernüberbleibsel zeigen sich Plasma-Anhäufungen, deren dicht angesammelten Körnchen zwei regelmässige sternförmige Figuren darstellen.* Die Strahle dieser Sterne werden durch die in gerade Linien aneinander gereihten Körnchen gebildet. Mehrere solche Linien reichen von einem Stern oder Anziehungscentrum in einem Bogen zum andern. Das ganze Bild ist äusserst klar, und erinnert lebhaft an die Art und Weise ausgestreuter Eisenstaub sich um die beiden Polen eines Magneten anordnet . . . . Ich schliesse mich ganz und gar der Sachs'schen Theorie der Furchung durch Anziehungs-Mittelpunkte an, nicht etwa aus theoretischen Gründen, sondern weil ich diese Attractionscentren *gesehen* habe."†

Thus the similarity of the cellular field to that of two unlike magnetic poles was recognised from the very outset. The figure in its highest development, as seen in Metazoa (figs. 1—5), has the character of a dumb-bell, whose spheroidal ends are termed "centrosomes," and with the rays they give off, "polar asters"; while the connection between them, of cytoplasmic fibres, is termed the "spindle." The astral rays diverge through apparently undifferentiated cytoplasm; but the spindle-fibres traverse or bound a clear space, apparently occupied by liquid during life. During the completion of the figure the nuclear wall has disappeared (fig. 1, E); of its contents the rod-like bodies, known as chromosomes, are disposed in a symmetrical star across or around the equator of the spindle. The chromosomes now split (fig. 2, G): the respective sister-segments of all diverge nearly simultaneously, E, and glide to the centrosome (which they may even enter) and then fuse into the daughter nucleus (fig. 2, I, J), the same process taking place simultaneously at either pole. The present study is devoted to the consideration of the character of the force operative in determining the cytoplasmic figure, which we term "mitokinetic force" (our reason for using this term rather than "karyokinetic force" will be found below, p. 564). The behaviour and influence of the chromosomes we pass over for the present.

---

* T. 24, f. 2h.

† "Die erste Entwicklung des Geryonideneies," 'Jenaische Zeitschrift,' vol. 7.

We must note that the figure as described is formed by growth; its origin in animals is in a minute granule, the "centriole," which becomes surrounded by a zone of clear cytoplasm, the "centroplasm"; this grows endogenously besides sending out from a peripheral "mantle-layer" rays into the ambient plasma, along which undoubtedly food-material passes. This centroplasm *has within it no trace of radial structure,* but consists of alveoli which enlarge as the structure grows (fig. 4). A second centroplasmic layer is formed within in contact with the centriole; and, as this grows, the alveolar layer of the original centroplasm becomes radially elongated, and affords a transition to a truly fibrillar radial structure; this is repeated a third time, whereupon the centriole divides with its inner centroplasm, and thus are formed the two "centrospheres" or "centrosomes" that constitute the ends of the dumb-bell of division. While the division of the centroplasm is taking place, the centroplasmic mass grows round the nucleus (in the oosperm the conjoined and appressed male and female nuclei); we may say that the nucleus becomes imbedded in the centroplasmic mass almost like an apple in a dumpling, so that the two centrospheres lie on either side of the nucleus.

The nuclear wall disappears (first at the two sides turned to the respective centrospheres); and the spindle is formed or rather completed by the extension of the inner rays across the nuclear space, while the rest of the nuclear contents are resolved into the chromosomes.

This is the history of the first segmentation-figure of *Rhynchelmis* (figs. 3—5), a worm allied to the common Blood-worm of our pond and ditch bottoms. I have selected it because the cytoplasmic figure is the largest known (1/125 × 1/100 = 0·20 × 0·25 mm.), and because it has been fully studied by Vejdovský and Mrázek; while, thanks to the kindness of the former, I have enjoyed the opportunity of studying some of the most important stages on the original specimens. In other cases, as in sperm-formation, a pair of minute centres united by a tiny spindle is seen on one side of the nucleus, and enlarges by the growth of all its elements except the centrioles: the spindle here seems to be bounded by a semi-pervious layer and to be turgescent as it grows. The figures (1, 2) copied from Wilson show a process of this type.

Again, in Higher Plants the poles have, as a rule, no visible existence except as indicated by the point to which the spindle-fibres converge at either end. These arise as a sort of radiation around the nucleus, extending from the whole of its periphery into the cytoplasm: the radial fibres come to approximate at their distal ends into bundles tapered at their ends, and ultimately in this way coalesce into the single spindle. The disceding daughter chromosomes join up at the ends of the spindles to form the daughter nuclei. When cell division does not follow nuclear division, as in the embryo-sac and in the pollen-formation of Exogens, etc., the daughter nuclei remain for some time joined up by spindles.

Thus the peculiar phenomena of osmosis and growth of the Metazoan centrospheres can shed no light on the nature of the forces at work in the spindle itself, resulting in the discession of the chromosomal segments; since the latter phenomena are repeated in Plants, which have no centrospheres.

II.

The problem of the character of the forces involved has been before my mind for at least twelve years, and resolved itself into the investigation of the physical laws according to which the cell behaved in respect of the dumb-bell and spindle figure. I tried hard to induce friends expert in physics to take up the study of cytology for this purpose; but in vain. The publication in 1902 of a remarkable essay by Gallardo, summarising and extending the arguments on behalf of the views to which I leant, and including experiments more or less on the lines of those I had planned, showed how necessary was detailed work on these lines, and how much remained to be done. I found also that the physical text-books and original papers, to which my own reading and the hints of my friends had led me, did not deal with the special problems involved: notably differences of "permeability" in the cytoplasm, and the redistribution of substances in a heterogeneous mixture under the action of a centred force to which the medium was not uniformly permeable; while the necessary conception of "material chains of force" had not yet been even formulated by physicists.

I therefore commenced last autumn a series of experiments with models in methodical continuation of haphazard essays dating at least half a dozen years back. I have received much valuable criticism (for the most part destructive of my crude ideas) from many physical friends, and notably my colleague, Professor Bergin, M.A., and helpful guidance in matters both theoretical and practical, from my son-in-law, Mr. William Cramp, A.M.I.E.E. The photographs that illustrate this paper have been taken immediately under my direction by Mr. H. C. Booth, except fig. 5, which I took myself.

III.

Since the days of Fol many explanations have been put forth, for the most part supported by able arguments, and illustrated by ingenious models. These explanations may be classified into *mechanical*, assuming a "pull" or a "push," and *kinetic*, invoking more subtle "centred forces." The most able advocate of the "pull theory" is Boveri. We reject it because, though some of the threads attached to the chromosomes retract with them on their discession (figs. 2, H, 4)—which would equally occur under the action of centred forces—others remain stretched across from pole to pole; nay, in some plants the chromosomes merely glide along the threads without any of these being retracted. The "push theory" has even less to recommend it, despite the able pleas of Meves; for the spindle-fibres are extremely flexible, and show no such changes of form and place as should necessarily accompany a pushing action.

We turn, therefore, to the "kinetic" theories, of which again are two types. (1) Rhumbler and Bütschli have advocated the effects of surface-tension, osmosis, and diffusion-currents on the alveolate structure of the cytoplasm, regarding the fibres as the extreme effects of radial tensions due to such forces on the form and distribution of the alveoli; and Leduc has contributed a study of the fields of force produced by diffusion-currents of scarcely more than molecular magnitude. We shall see later that the conditions of the poles of the spindle are such as to render the action of such forces inadmissible to explain the cell-figure, nay, such as to prevent the formation of a spindle were the action of these forces propagated to a distance through the cytoplasm.

IV.

There remains only the supposition that the cell-force is of a "dual" character (to use Faraday's word) like electrostatic force or magnetism; that the cell-centres are the seats of opposite charges; that the fibres of the spindle (and of the asters, when such are present) are differentiated in a "field of force," and transmit lines of force under the action of a pair of "unlike" poles. This conception, present, as we have seen, to Fol at the very birth of modern cytology, has been powerfully advocated by many, notably by Giard* and

---

* Giard's remarks—as early as 1876—are of singular interest, indicating as they do the lines on which investigation should be profitably conducted. They occur in a *résumé* of his course at the Faculty of Lille, under the title "L'Œuf et les Débuts de l'Évolution," communicated to the editor of the 'Bulletin Scientifique du Nord' at his request, in order to give his readers some idea of the new discoveries and conceptions in the domain of reproduction and cytology.

He writes: "L'explication physiologique du phénomène . . . . doit être evidemment cherchée parmi les phénomènes physico-chimiques et la production de pôles électriques ou by Ziegler,* who devised an ingenious magnetic model, too simple, however, to carry theory much further; and especially by Gallardo,† whose electrostatic model was a great step in advance. It consisted in the introduction of the poles of an electrostatic machine into a glass trough containing sulphate of quinine suspended in oil of turpentine. The quinine segregated out in filaments, or chains, forming a spindle between the terminals, so that the behaviour of "chains of force" (see p. 555) might be studied. He also showed that the introduction of a third terminal put to earth produced a deviation of some of the fibres from the belly of the spindle to itself—the figure being, in fact, a "triaster," such as sometimes occurs in dividing cells.

The adverse criticisms on Gallardo's paper showed clearly that biologists for the most part had but ill-defined ideas of the action of dual forces, and the application of their laws to the conditions of the cell. As mentioned above, the necessary conception of the "material chains of force" had not even been stated by the physicist; and the conception of differences of permeability had not yet been applied—even by Ziegler and Gallardo—to cytological substances. These were first put forward in my Preliminary Note "Des Chaînes de Force, et d'un nouveau Modèle magnétique des Mitoses cellulaires."‡

V.

To render the actual experimental study intelligible to the biologist, we must review the conception of "lines of force" and its two extensions just mentioned. Faraday, unable to admit the possibility of action at a distance, suggested that wherever such appeared to take place between two bodies or centres, the intervening medium must be in a state of strain: the direction of the stresses and consequent strains centering round the interacting bodies, and being continuous from one to the other along lines which he termed "lines of force."

Clerk Maxwell, who much extended the idea, gave this definition :—"If we

---

électro-magnétiques dans le noyau. Peut-être arrivera-t-on à mettre expérimentalement en évidence ces curieux processus en employant des sphères liquides en suspension dans un autre liquide, comme le faisait Plateau, mais en mélangeant ces liquides de substances fortement magnétiques et capables d'acquérir des pôles sous l'influence d'aimants puissants. Il y aurait tout un ordre de recherches à entreprendre dans ce sens." ('Bull. Sc.,' vol. 7, 1876, p. 258.) I think it right to cite this passage, as the periodical is not widely circulated in England, and the passage, to my knowledge, is rarely quoted ; I blush to say that I first read it in Gallardo. It clearly foreshadows the line of work pursued by Gallardo and myself.

* "Üntersuchungen über die Zelltheilung," 'Verh. Deutsch. Zool. Ges.,' 1895.

† "Gallardo, "Essai d'Interprétation des Figures karyokinétiques," 'Ann. Mus. Buenos Aires,' 1896 ; "Interprétación dinámica de la División Célular," 1902.

‡ 'Comptus Rendus,' June 10, 1904.

draw a line such that at every part of its course it is coincident in direction with the force at that point, this line may be called a 'line of force,' since it indicates the direction of the force at every part of its course."* It will be noted that such a line is a geometrical line, not a material line. Maxwell also recognised that for a field of two "unlike" poles the lines have the distribution of stream-lines in liquid in relation to an upwelling source and an engulfing sink, and, like Kelvin, saw that this distribution was identical with the lines of the flow of heat in a conductor between a source of heat and a refrigerator; while the field of two "like" poles corresponded with the thermal field of two sources of heat, or two refrigerators, as the case might be.

A *quantitative* meaning has been attached to the conception of "lines of force," and with this addition it now forms the base of the proximate or practical theory of electrical engineering. I can best illustrate this quantitative connotation by the use of an analogy which I have myself found helpful. We may attach such a quantitative meaning to the "ray of light" in geometrical optics, by defining the intensity of illumination of a surface as the number of "unit rays" falling on it at right angles (or their equivalent at other angles) per unit of area. Adopting this convention, we pass to the question of "permeability."

We find that if the medium traversed by lines of force be not uniform, the lines of certain forces travel more readily in some substances than in others. Thus magnetic lines pass more readily through soft iron than through air, through air than through bismuth; similarly electrostatic force travels more readily in sulphur than in air, etc. Such differences are termed differences of "permeability" in relation to magnetism, of "specific inductive capacity" for electricity†; but the term permeability has already received a wide general application; and we shall use it in relation to mitokinetic force, without making any assumption as to its nature. The "permeance" of any portion of a field of force is defined as the product of the permeability of the material into its cross section, divided by the length of the path travelled by the lines.

---

* 'Sci. Works,' vol. 1, p. 467.

† [I find that the term was indeed introduced into Physics in this wide sense by Lord Kelvin in 1872: "The common word 'permeability' seems well adapted to express the specific quality in each of the four analogous subjects. Adopting it we have thermal permeability, a synonym for thermal conductivity; permeability for lines of electric force, a synonym for the electro-static inductive capacity of an insulator; magnetic permeability, a synonym for conducting power for lines of magnetic force; and hydrokinetic permeability, a name for the specific quality of a porous solid, according to which, when placed in a moving frictionless liquid, it modifies the flow."—"A Mathematical Theory of Magnetism," in 'Papers on Electrostatics and Magnetism,' Section 628.—Added during the printing.]

Now the laws of distribution of the lines in a magnetic field are these:—1. All lines travel from one pole to another of opposite sign, in continuous curves, which may, however, only be completed at infinity. 2. The number of lines in any part of a field is proportionate to the permeance of the path they have to travel. The terms "reluctivity," "reluctance," are defined as the reciprocals of permeability and permeance respectively; it is often more convenient to use them, and to speak of a material as of "high reluctivity" than as of "low permeability."

VI.

The path traversed by these lines in air can be approximately shown in the case of magnetism by placing a piece of smooth paper horizontal in the field to be studied, strewing it with magnetic dust, such as iron filings, reduced iron, or powdered magnetic oxide of iron (Fe_3O_4)* and gently agitating the paper, when the powder is seen to arrange itself in the well-known curves. To obtain such delineation in fields with varied poles I found it necessary to devise a special apparatus, which has been constructed for me by the Crypto Works Company, Ltd. (now the Crypto Electrical Company).

A square base-plate of cast iron supports four columns of brass, screwed at their upper ends, and fitted with large flat nuts. Vertical electromagnets are provided, with the soft iron cylindrical cores longer than the coils, and rounded above to give more horizontal spread to the lines leaving them: they rest on the base-plate, which serves to lessen the air-gap; and is, for a pair of straight electromagnets with opposite poles, the equivalent of the yoke or cross-piece of a horseshoe-magnet. The extra length of the cores is a device to secure stability: either the top of the core passes through a hole in a perforated slab of wood supported on the brass columns; or the coil rests on a perforated cylinder of lead through which the lower part of the core passes, in which case a thin plate of brass may replace the wooden slab. The latter arrangement allows any distance to be put between the poles, instead of the limitations imposed by the perforations of the slab. The coils are attached at the lower end by flexible connections (of lamp-cable) to the binding-screws of a mercury commutator, which allows of the ready reversal of the polarity of any magnet singly. An ammeter, a rheostat, and a source of constant current at 4 volts complete the apparatus. The coils will stand for a short time a current of 5 ampères as a maximum.

---

* I use the native magnetite, crushed, levigated, separated from impurities by magnetism, and dried; it is much blacker than metallic iron, and the figures show up better.

The perforated slab or brass plate serves as a "stage" on which we can place our paper; or, if we wish to delineate our field in a liquid medium, a glass plate is placed on the paper, which now serves to reflect the light, better illumination being thus obtained than by the use of porcelain. To delineate the field with dust in air, I sprinkle the dust on the paper by shaking it through fine copper gauze covered by a cambric rag; and for tapping I use the "connection" of a bicycle inflator—consisting of two metal nuts united by thick rubber tube—which is convenient as enabling me to regulate the taps with great delicacy. It is often best to tap the base or columns of the apparatus rather than the stage or the paper. For the study of the "chains" in liquid I use a mixture of the magnetic dust with glycerine, dissolved or melted balsam, or melted jelly, spread on a glass or porcelain plate. Under the influence of the field the liquid soon differentiates into chains of the magnetic material, separated by clear spaces, and the effects of the continuance or increase of the magnetic force can be studied.

A Leitz "large microphotographic apparatus," provided with a 5-inch RR lens, and with the camera vertical, serves to record the figures obtained; the magnetic apparatus being placed on the base-plate of the photographic instrument.

VII.

We can now study the nature of the figures obtained by our apparatus, and we begin with the dust-paper results. While resting on the paper, the dust is kept stationary by friction; but, on tapping, it rises in a cloud into the air, and is free to respond to the magnetic stresses present, and to arrange itself into the material curves which we term "*chains of force*," and define as the "files of more permeable substance segregated along lines of force out of a mixture under the stresses of a centred force": in this case the mixture is that of air and dust in the cloud. Faraday, indeed, already noted that the presence of the iron dust was a disturbing factor in the distribution of the lines of force in air which he employed it to demonstrate; but the first explicit recognition of such chains as a system to be studied is, I believe, to be found in the preliminary note cited above.

By the laws of the distribution of lines of force, such a chain of particles, possessing n-times the average permeability (taking into account the air intervening between its particles) of an adjacent tube of air of equal section, will contain n-times as many unit lines of force approximately.

The properties of these chains are far better studied when formed in a viscid medium, which acts as a bond between their successive particles. Here their toughness is remarkable. With a permanent magnet I have obtained horizontal spindles in glycerine in a vertical trough which, despite their sag, persisted for three days; but for such results we must, of course, use dust of special fineness. In such a medium the chains may be seen to sag without rupture under the influence of gravity; they can be pushed aside by contact with a glass rod, or swayed by currents; or, if the vessel that contains them be moved, they may be deflected by the shifting of their position relative to the poles before they rupture, through the lag imposed by the viscidity of the medium (fig. 14). Owing to the relatively high permeability of their substance, they retain in all these cases of deviation, despite their elongation, the majority of their lines of force: for, under the conditions of the experiment, the elongation is of a lower order of magnitude than the ratio of their average permeability to that of the medium.

VIII.

Under the microscope a chain is seen to be *spongy*, composed of loosely laid strands joining at very acute angles. Its constitution is conditioned by three factors: (1) The relative permeabilities of the powder of the medium; (2) the viscosity of the medium; (3) the intensity of the magnetising force: as the last increases, the texture becomes more serried by the squeezing out of the intervening liquid. As we have just seen, the chains are composed of fine strands meeting at acute angles, so in the segregation to form the chains themselves two of them may meet and unite at an acute angle—"anastomose," in fact. Such junctions are to be found in almost all published figures of dust in air on paper from the time of Faraday (figs. 10, 13, 15); but they are especially easy to observe when the dust is suspended in liquid (figs. 6—9). Once formed, the magnetic stresses hardly tend to rupture them, and they are nearly as resistent as any other part of the chains (if not quite): they are often sufficiently abundant to give the whole figure the aspect of a network with rhomboidal meshes. Of course, *lines of force* cannot anastomose in this way, and the existence of anastomoses in the cell-figure was necessarily a difficulty in the way of explaining that figure by centred forces, so long as the distinctive characters and properties of the *chains of force* were not recognised.

In a viscid medium, moreover, the chains of force are often seen to cross one another at slightly different levels—to interlace in fact—simulating such true networks as we have just seen. Here is another point of difference between *chains of force* and *lines of force in a uniform medium.* Now crossings as well as networks are of frequent occurrence in the cell-figure, notably when it is in process of formation, and before the spindle has reached its full development. At this stage the astral rays growing out from either centre will frequently extend beyond the equator of the cell, into what we may term the "domain" of the other centres here such crossings are especially common, and occur when the direction of the rays is approximately straight. From this we may infer that the reluctivity to the mitokinetic force of the undifferentiated protoplasm and its inclusions is very high, so that the rays are but little affected by that centre to which they are not as yet organically attached. As soon as two rays from opposite centres actually meet and join, their straightness passes into a curve concave to the intercentral axis.

The comparison between the cellular and the magnetic chains can be but an imperfect one at best, on account of the different ways in which they are formed respectively: the magnetic chains are formed simultaneously* by segregation along their whole course, and the cell-chains by progressive outgrowth from the centres. All the same, we have seen that the crossings and interlacings, which have been invoked as incompatible with magnetic analogies, are actually produced in our magnetic model, and their presence in the cell-figure is no argument against the kinetic nature of the cell-spindle, or the dual nature of the cell-stresses. Take, on the other hand, the assumption that the fibres are the transmitters of a mechanical push or pull: they are imbedded in viscid protoplasm, so that by friction they would communicate their motion thereto. Thus, where crossing occurred, the motions of the adjacent crossing fibres would be mutually transmitted, and would effect lateral displacements which have been neither figured nor recorded, and which it is safe to conclude do not exist in the cell. In this respect, therefore, the crossings of the astral rays tell equally against both of the mechanical explanations of the cell-figure, while they are actually reproduced in our magnetic model.

IX.

In certain cellular fields, notably in the segmentation-cells of the Slug (*Limax*) described and figured by Mark in 1879, the cell-fibres diverge in spirals from the poles. This is one of many proofs that forces other than, and additional to, the centred mitokinetic force, are operative in the dividing cell. Here, again, we see the contrast between the behaviour of *lines* of force in a homogeneous medium and our material *chains* of force. If we rotate in our field of two unlike poles the glass plate covered with viscid mixture, we see the chains become sigmoid or ʃ-shaped, owing to the lag imposed by the viscidity of the medium (fig. 14); it is interesting to see how they often become ruptured into a series of short lengths which individually lag more than the chains as a whole. Such figures represent approximately the axial section of a bipolar field, of which the polar areas are subjected to equal and opposite torsions. We use melted jelly or balsam to obtain a figure whose changes during revolution are arrested by its solidification, so as to allow of its being preserved and photographed. The interlacings of chains are especially well shown in some of these "torsion" figures.

---

* I speak only of the conditions under which I have experimented. If the poles were remote, and the magnetomotive force long maintained, I should not doubt that the magnetic chains would grow out from the poles.

X.

The distribution of the chains of force in the cell is very imperfectly represented by the classical figures of the magnetic field of two "unlike" poles hitherto utilised; for these always depict the axial portions of fields of *indeterminate* extension. They have hence the character that the lines tend to pass from one pole to the other without deflection, and so form a series of curves all concave to the interpolar axis—the straight continuations of this axis being supposed to meet at infinity. Now the chains in the cell are differentiated into (1) "astral rays," radiating more or less directly outwards from the centres, and usually nearly straight or even *convex* to the interpolar axis, but concave to its prolongations; and (2) the spindle-fibres extending from pole to pole, and concave to the interpolar axis.

We can make a far more exact magnetic model than the common one, if we limit the field by an envelope of highly permeable material; for which purpose I use a sheet of charcoal-iron with an oval window, like a photographer's vignette-mask. We now see the chains radiating from the pole outwards, straighten out, or even become *convex* to the interpolar axis and concave to its prolongation, thus turning their backs on the spindle-fibres (figs. 7, 8, 15). In the cell the rays appear straight most frequently, but the back-turned (*adossé*) condition is often seen and figured. The analogy of this model would lead us to infer that the "Hautschicht" of the cell is highly "permeable" to mitokinetic force.

A still closer approximation to the character of the mature dumbbell figure of the animal cell is seen in glycerine or other viscid media, when we allow the magnetic stress to continue after the first segregation has taken place (figs. 9 and 10). The chains, like other permeable bodies, tend to place themselves in the strongest part of the field. Consequently, the interpolar chains move laterally in towards the axis, shortening up as they do so by the squeezing out of the liquid in the interstices between consecutive particles and adjacent strands. Since this action lessens as we recede from the neighbourhood of the axis, we reach on either side a zone where the viscidity of the medium is strong enough to resist it, and to inhibit the motion of the chains. Thus the spindle becomes thickened and denser from the lateral crowding in of chains (fig. 10) while peripherally there is left a clear space from which the chains have passed inwards; the result being a clearer differentiation into central spindle and polar asters. Our figures show how much closer an approximation we have obtained in our model to the cell-figure by combination of the two devices, (*a*) the provision of a permeable envelope, and (*b*) the production of the figure in a medium where the prolonged action of the stresses may have its due effect. The clear space has been recognised in the cell, and received the name of "Bütschli's Space" (Rhumbler). We can reproduce the same effect on smooth paper if the tapping be sufficiently energetic or prolonged: it has doubtless been often obtained involuntarily by the physicist, who has evidently not thought it worth his while to reproduce a "failure" so far as his object was concerned; for that has merely been the delineation of the direction of the lines of force in a uniform medium of indeterminate extension.

XI.

In certain cases, more than two centres appear in cells, united by spindles, and constituting "tri-," "tetr-," and "poly-asters." It has often been laid down as a mathematical truth that the action of a dual force is incompatible with the formation of a field with an odd number of centres all joined by spindles. Rhumbler definitely states as a law: "Magnetic lines of force can never form three spindles between three consecutive poles . . . three adjacent poles even when there are more than three poles in the field, can never be joined up by three spindles"*; and his argument would certainly apply to all dual forces. There is, however, a flaw in his exposition: as Gallardo had pointed out some years before, two opposite poles, and a third point at zero, behave as three distinct centres in his electrostatic model (above, p. 552). We may call two unlike magnetic poles "positive" and "negative" respectively, when a mass of soft iron will correspond to a "zero" centre. Thus we place below our stage two unlike magnetic poles at the ends of the base of an obtuse-angled triangle, and a core (without a coil) at the vertex, with a wafer-like disc of iron lying on the plate above; the figure obtained is seen to be a triaster with its centres joined up by consecutive spindles (fig. 8). The higher the permeability of the chains, the greater the reluctivity of the medium, the more nearly could we approach an equilateral triangle; as it is, in the cell triaster as figured by many observers the three centres are rarely, if ever, equidistant.

---

* "Mechanische Erklärung der Ähnlichkeit zwischen Magnetischen Kraftliniens systemen und Zelltheilungsfiguren," 'Arch. Entwicklungsmech," vol. 16, 1903. "Von magnetischen Kraftlinien können niemals drei Spindeln zwischen drei Polen hervorgebildet werden . . . . auch wenn mehr als drei Polen im Diagramm vertreten sind, können niemals drei benachbarte Polen durch drei Spindeln verbunden sein" (p. 482).

Again, we can obtain a tetraster with four consecutive spindles and a fifth spindle uniting two opposite centres, like that often found involving four nuclei in the embryo-sac of Flowering Plants, by using two unlike poles alternating with two cores (fig. 9). This figure is clearly the preceding triaster doubled, or, as we may say, united with its "reflection." We can obtain the equivalent of four triasters joined into a square, if we place four alternate poles at the angles, and a core in the centre (fig. 10). Either of these two figures can by addition of suitable centres be extended indefinitely to "fill space." Thus, if we replace the term "pole" by "centre" both of Rhumbler's dicta fall to the ground: the physical explanation is obvious, and there is no jugglery. It would appear that in the cell, when there is a plurality of centres, they tend to acquire charges such that the number of spindle-connections is a maximum.*

XII.

The tetraster, which occurs, for instance, in the spore-mother-cells of the Hepaticæ, where four centres occupy the poles of a tetrahedron, and the spindles extend only to a central mass of chromosomes, may be modelled in a plane by a set of three "like" poles surrounding a central core or an opposite pole (or by a single central pole and three cores, which, of course, gives a weaker field). The addition of a fourth "like" pole vertically above the core would complete the model, supposing that the core could also rise to the centre of the figure, drawing up with it its three spindles. Or we may model the tetraster in a plane with four "like" poles and a central core or "unlike" pole to represent the aggregated chromosomes, or a central pole and four surrounding cores (fig. 11). The chromosomes are highly permeable to mitokinetic force (see below, p. 564).

XIII.

We can study and elucidate other cell-figures by the aid of models. Thus Vejdovský and Mrázek have found that one or both of the centrosomes may be drawn out into a spheroidal "blob" on the side next the attachment of the spindles—a state of things evidently due to the opposite actions (*a*) of the spindle under mitokinetic force, and (*b*) of the tractive forces in the cytoplasm pulling the centrosomes towards the periphery of the cell (fig. 4).

---

* This point will be further discussed in Part II.

We can model this by putting a heap of heavy, coarse powder just in front of the poles, or by cementing to the glass a small disc of charcoal-iron in the corresponding place, especially if we cement a larger disc immediately over the pole (fig. 15). I also figure a "monaster" from Boveri, where the centrosome has broadened out, but failed to divide (fig. 12). Its magnetic model is thus obtained: a blunt crescent of charcoal-iron is cemented over the pole of a single magnet to represent the centrosome, and a small disc in its concavity represents the nucleus (fig. 13). From the correspondence of the distribution of the chains of force, we must infer that the *nucleus*, or at least the *nuclear wall is highly permeable to mitokinetic force.* This is a very good illustration of the value of our method of studying by models, in enabling us to gain an insight into the laws of the cellular field.

XIV.

We are now sufficiently familiar with the essential conceptions of the theory; and can profitably examine in detail how far such forces as osmosis, surface-tension, etc., could be used to explain the cell-figure. Thanks to the careful researches of Vejdowský and Mrázek on the most favourable object known, we are aware that the osmotic and surface-tension actions at the two cell-centres are of exactly similar character. They appear (*a*) to be limited to the immediate neighbourhood of the centrosomes, (*b*) to have for their object the nutrition of these, and (*c*) not to be transmitted to a distance. The first-formed rays, indeed, seem different in their behaviour to the later ones, and to have no kinetic action, no inductive results on those of the opposite poles. Were there any action at a distance the field produced must resemble that of two "like" magnetic or electrostatic poles. For if "like" centres act through a permeable medium, (1) a particle free to move under their influence, if on the axis joining them or its prolongations, will tend to move along that straight line to the nearer one if the force be one of attraction, away from it if it be one of repulsion; except that if it be at the centre of figure (assuming that the forces are equal in magnitude) it will stay there. Thus the line drawn through the poles is a "line of force." (2) Any point on the equator, or plane bisecting the interpolar axis at right angles, will tend to move to (or from) the centre of figure; so that every line on the equator passing through the centre is also a line of force. (3) The remaining lines of force all diverge from the poles, and are convex to the interpolar axis. The figure of the distribution of the lines of force in such a field we may call the "anti-spindle" or "crossed field." Thus the formation of a spindle is impossible between two poles of "like" or of undifferentiated character.

As stated above Leduc has demonstrated this for diffusion-currents of scarcely more than molecular value.* If we place in a solution of a substance a crystal of the substance dissolved, this will determine a flow of liquid towards itself, and may be termed "positive"; if we introduce a droplet of the solvent, it will act as a centre of repulsion, or "negative" pole. If we take a solution of saltpetre, and introduce into it a crystal of this substance, and, at a distance, a droplet of defibrinated blood—of lower isotonic value than the solution—the blood-corpuscles may be seen to flow along the curves of a spindle to the crystal. Two "like" centres, whether of concentration or of dilution, always behave as "like" poles, and determine the "crossed field"; consequently this group of phenomena cannot determine the cell-field. I should say frankly that Leduc's experiments were undertaken in the hope of applying the explanation of diffusion to mitokinetic force; so far as I know, he still retains the belief that it can be done.†

Bütschli has described and photographed‡ alleged "spindles" in films of jelly cooled and dried on glass, around air bubbles, thus converting the alveolar structure of the jelly, which he has done so much to elucidate, into a radial structure. Now, on this argument, *a posteriori*, we may note:—(1) His figures appear in his plates absolutely ambiguous, the rays of some of the spindles meeting at an angle on the equator, as if the medium were not permeable, and the far pole had no inductive effect. (2) The centres, as figured in the less ambiguous cases, are not such as to carry the conviction that they are "like" members of a pair, and, though the differences *may* be due to differences of level and focus, it is rather significant that Bütschli does not notice their unlikeness, or think it needful to comment on it. (3) The discussion in the text is wholly inadequate, showing clearly how far the author has failed to realise the physical difficulties in the way of his views. I may translate, in illustration of this physical inadequacy, the opening passage, describing the asters, and leading up to the spindles: "The tension exerted in all directions by the decreasing size of air-bubbles on the surrounding alveolar network must, of course, decrease in the inverse ratio of the square of the distance. The decrease of the visible action of this force in the transformation of the alveolar into a radiate structure must, however, *since it involves relations of volume,* take place in the inverse ratio of the *cube* of the distance."* We know nothing of forces acting "inversely as the cube of the distance," and I cannot see why Bütschli should have thought it necessary to assume such.

---

* "Champs de Force de Diffusion," 'Comptes Rendus Assoc. France,' Montauban, 1902, p. 309; "Les Champs de Force chez les Êtres vivants," 'Comptes Rendus Soc. Biol.,' vol. 4, p. 367, 1903.

† [Leduc has now recognised that the centres in the dividing cell are "like" centres *in respect of diffusion*, and explains the cell-spindle as a false-spindle, 'C. R.,' December 5, 1904: this explanation is irreconcileable with the existence of continuous spindle fibres extending in the cell from pole to pole across the equator.—Note added during the printing.]

‡ "Untersuchungen über Strukturen, insbesondere über Strukturen nichtzelliger Erzeugnisse des Organismus, und über ihre Beziehung zu Strukturen welche ausserhalb des Organismus entstehen," 1898.

Rhumbler, who also believes that the action of surface-tension and diffusion on the alveoli of Bütschli produces the cell-figure, has devised an ingenious model to illustrate the action of the forces at the centres.† He forms a net of some 300 india-rubber rings each united to its neighbours by six bonds to form a hexagon; the net is approximately circular, and some 20 meshes across. This is stretched on an iron hoop over a sheet of cardboard or paper in which holes are pierced. Through these holes the net can be drawn down to represent centres of radial attraction. If two such centres be taken and the net pulled through both, the intervening meshes along the intercentral axis are pulled out and narrowed, the narrowing being extreme at the poles, and least half-way between them. He interprets the widening of the central row of meshes towards the equator as showing that under these conditions two undifferentiated or "like" forces can produce a "spindle" field.

I confess that I was not a little puzzled by this apparent contradiction of mechanical principles. However, on looking at the net, we find that the so-called "spindle" arrangement involves about eight meshes in length and ceases completely within two meshes on either side of the centre; and that the long sides of all the remaining meshes form broken curves, which, with a minimum of smoothing out, clearly follow the lines of the "crossed field" just as might have been anticipated. The apparent "spindle" is a mere grain- or texture-result, due to the large size and inadequate number of the meshes. Had he made this net of 2700 meshes, so as to have a diameter of nearly 60 meshes, and put his centres 24 meshes apart, he would have seen that the "spindle" was no wider than before. Yet even with this enlargement the number of meshes in the intercentral "spindle" would have been of a much lower order of number than the Bütschlian alveoli in the spindle of a dividing cell; while the cell-spindle is much wider relatively to its length.

---

* "Die Zugkraft, welche bei der Verkleinerung der Luftblasen allseitig auf das umgebende Wabengerüste wirkt, muss natürlich umgekehrt proportional dem Quadrat der Entfernung abnehmen. Die Abnahme der sichtbaren Wirkung dieser Kraft, welche sich in der Umformung des Wabengerüstes zu strahliger Beschaffenheit äussert, nimmt dagegen wohl, da es sich um körperlichen Verhältnisse handelt. umgekehrt der 3 Potenz der Entfernung ab," p. 158.

† "Versuch einer Mechanische Erklärung der indirekten Zelltheilung: 1 Th. Die Cytokinese," 'Arch. Entw.,' vol. 3, 1896.

Consequently, as I have shown elsewhere,* the model does not justify the theory of its author. His apparent triaster is founded on the same error.

Though the scope of the present study is essentially limited to the achromatic figure, I must say a few words on the chromosomes. If we represent the split chromosomes by strips of charcoal-iron, we find the distribution of the magnetic chains corresponds to those of the cell-figure, and may hence infer that the chromosomes are permeable to mitokinetic force, and consequently susceptible to its induction. The result of this is that two adjacent segments will be the seats of like charges, and repel one another: any slight movement that brings the two sister segments so that they are respectively nearer opposite poles, will tend to separate them by the effects of this induction, and the segments will then recede from one another. As they do this, any chain connecting the segment with the nearer pole will shorten and thicken. It thus simulates the behaviour of a muscular fibre, but differs in that it shortens up to nothing, *i.e.*, it is taken up into the centre of force or disappears into the cytoplasm: such fibres have been termed "tractive" fibres *par excellence.*

I have begun a series of experiments using aggregations of magnetic matter free to move in a viscid medium to represent the chromosomes and diffused dust to form the spindle fibres. Here we can see the magnetic chains attached to the aggregates move up as the aggregates move towards the poles; we cannot call such shortening magnetic chains "tractive," and have no right to ascribe this function to the corresponding fibres in the cell. But my work on the models of the chromosomes is too inchoate for me to do more than indicate the lines on which it is shaping. I should add that in 1898 I definitely wrote:—"The splitting of a viscid thread is one of the most difficult mechanical feats to accomplish. Suppose, then, that there is a certain polarity about the granules of chromatin, through which, after their division, they tend to recede from their fellows as far as possible: through this they will determine a splitting of the filament on which they are strung."†

XVI.

The name I have given to the dual force we have just studied may perhaps be criticised on the ground that, as "karyokinetic force" is a more or less familiar word to cytologists, there was no need to invent a new term.

---

* 'Arch. Entwickl.,' vol. 19, 1905.

† "Nuclear Reduction and the Functions of Chromation" ('Nat. Sci.,' vol. 13, and 'Biol. Centr.,' vol. 18, 1898). Ralph S. Lillie has developed similar views, and regards the force as definitely electrostatic. "On Differences in the Direction of the Electrical Convection of certain Free Cells and Nuclei," 'Amer. J. Physiol.,' vol. 8, January, 1903; see also 'Biol. Bull.,' vol. 4, 1903.

The fact is that the etymological meaning of "karyokinesis" (= disturbance of the nucleus) forbids its correct application to the dual force we have investigated. For this reveals itself first in the *cytoplasm,* while the nuclear wall is still intact, and since this wall is highly permeable, it must act as a screen between its contents and the force in the cytoplasm, just like a spherical investment of charcoal-iron would screen all within it from external magnetism.

If by a permissible extension of meaning we use *karyokinesis* as an inclusive term to designate *all* the phenomena of "indirect cell-division," we find that this covers not only the dual force, but the forces that separate the poles, and possibly others as well, for the process is not a simple one. Hence, for this dual force under which the cytoplasm gives rise to chains of force in the form of the dumb-bell figure, comprising spindle and asters, we have had to use the term "mitokinetic," signifying the *kinesis* which reveals itself by a *thread*-structure.

Summary.

1. The cytoplasmic figure of the dividing-cell is a strain-figure, under the action of a *dual force,* analogous to magnetism, and still more to statical electricity; without prejudging its nature further we may call it "mitokinetic force" or "mitokinetism."

2. The conception of *relative permeability* is invoked for the first time in elucidating the behaviour of this dual force in comparison with those of magnetism.

3. By comparison with magnetic models we find that (*a*) the spindle fibres and astral rays, (*b*) the Hautschicht of the cytoplasm, (*c*) the nuclear wall, and (*d*) the free chromosomes along the cell-spindle must all be of high permeability to mitokinetism as compared with the other structures of the cell (see also 7, 12, 14).*

4. As the cell-structures are all *material,* the conception of *geometrical* lines of force is inadequate to explain them. We must recognise that the effect of stresses within a mixture of substances which are of different permeability and free to rearrange themselves, will be to segregate out the more permeable material in strands along the lines of force whose distribution they modify. Such strands we may call "*material chains of force,*" or for brevity simply "*chains of force.*" It is only our recognition of these structures that enables us adequately to utilise electric and magnetic models for our explanations.

---

* [3*a*. The periphery of the centrosome is also highly permeable, since it screens the alveolar contents from the action of the surrounding field (see fig. 4).—Note added during printing.]

5. The fibres of the cell-spindle and astral rays are of the nature of chains of force; their *production by outgrowth* of differentiated cytoplasm from a centre is different to that *by simultaneous segregation* of the magnetic chains in a mixture of magnetic dust and liquid, etc., under the condition of the experiment (but see note to p. 557). We can, however, utilise the study of the physical properties of magnetic chains, and apply by analogy many of the results to the cell-chains.

6. Thus, magnetic chains of force (*a*) *anastomose* and (*b*) *interlace* at adjacent levels as cell-chains are found to do.

7. The straight or backturned radiations of the astral rays are repeated in the magnetic models if we put in place of the outer cytoplasmic membrane (Hautschicht) an envelope permeable to magnetism (see 3, *b*).

8. A disc of iron put in front of a crescentic pole-piece gives the same field as the nucleus in the hollow of a single crescentic centrosome (see 3, *c*).

9. The axial section of a *spiral cell-field* may be modelled magnetically if we use a mixture of magnetic dust in a viscid medium, and rotate it over a pair of unlike poles. From this we infer that other forces than mitokinetic are operative in the dividing cell, where such figures are seen.

10. The *separation of the poles* of the cell figure must be also due to other forces, and the effect of their traction against that of the spindle is sometimes exceptionally well seen in the occurrence of "*blobbed*" *centrosomes.*

11. We can obtain a *triaster* (and combinations implying the triaster) of three poles joined by consecutive spindles by the action of three centres, two poles of opposite sign, and the third of zero sign—in the models the core of a magnet without a coil. These figures may be extended by additional centres to "fill space."

12. In most multipolar cell-figures the poles are so distributed as to produce the maximum of spindle connections.

13. We can produce a figure of four poles united by spindles to a common centre of opposite sign or at zero; this is the analogue of the tetraster about a central mass of chromosomes (see 3, *c*).

14. Chains of force, like movable magnets, tend to move into the strongest part of the field; thus if we form a magnetic spindle in glycerine, the lateral chains tend to sway or drift in towards the axis, becoming shorter and denser, the clear lateral space from which they have receded corresponds to "*Bütschli's Space*" in the cell.

15. The separation and discession of the sister segments of the chromosomes from the equator to opposite poles is such as would take place under the action of a dual force, and can be reproduced in our model (see 3, *d*).

16. A spindle figure can only be obtained in a field with the two unlike

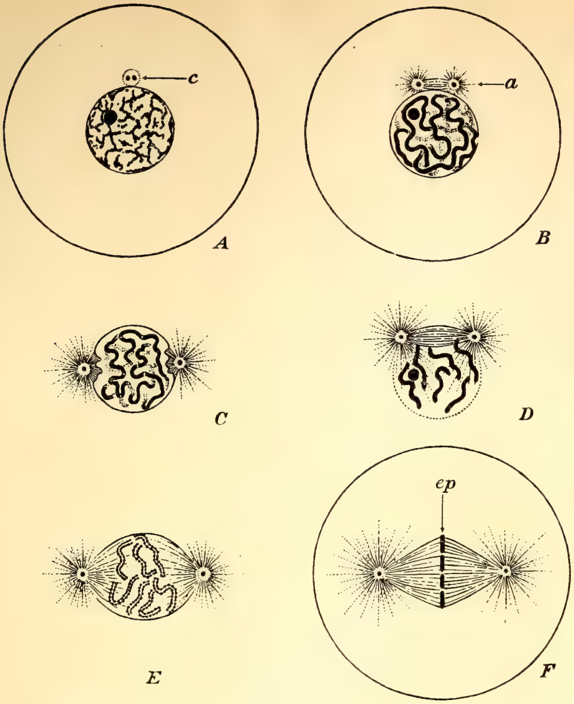

c
a
A
B
C
D
ep
E
F

FIG. 1.

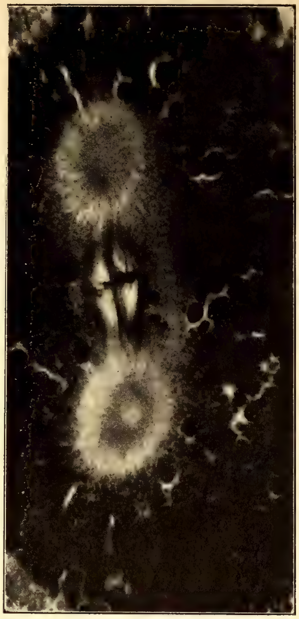


FIG. 5.

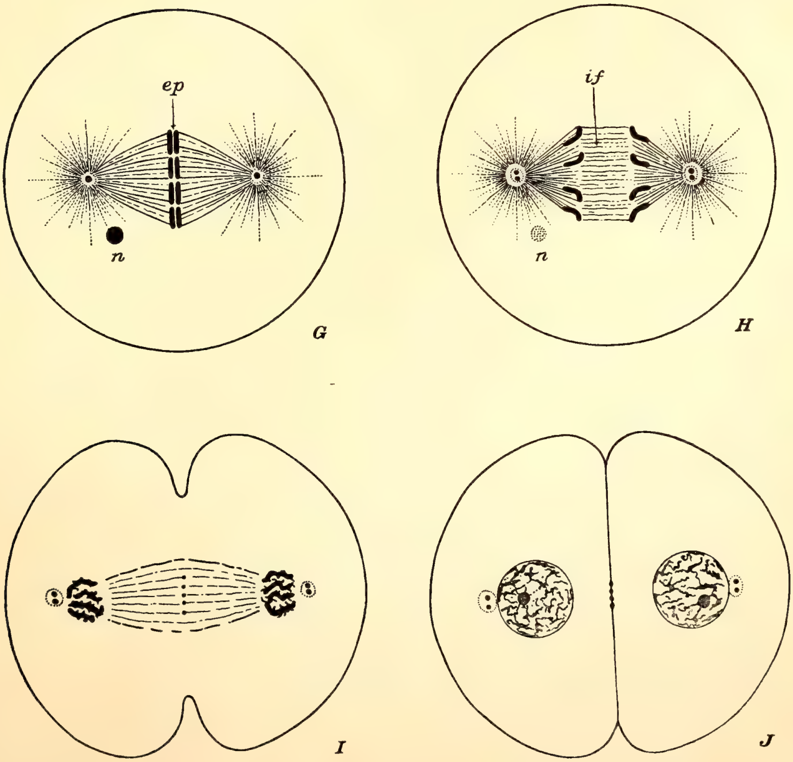

ep
n
G
if
n
H
I
J

FIG. 2.

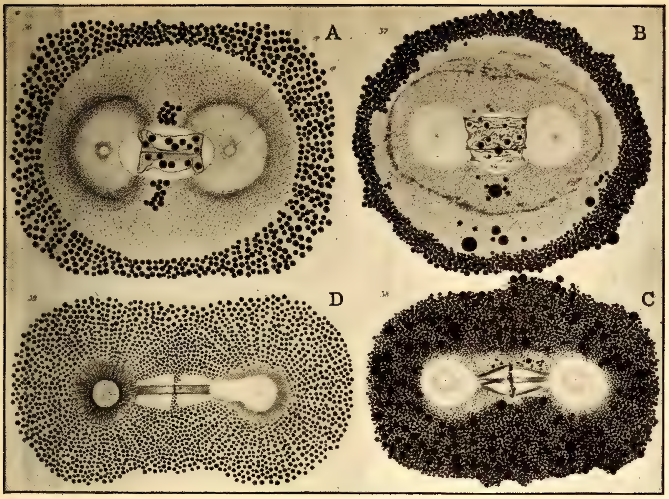

36
A
37
B
39
D
38
C

FIG. 3.

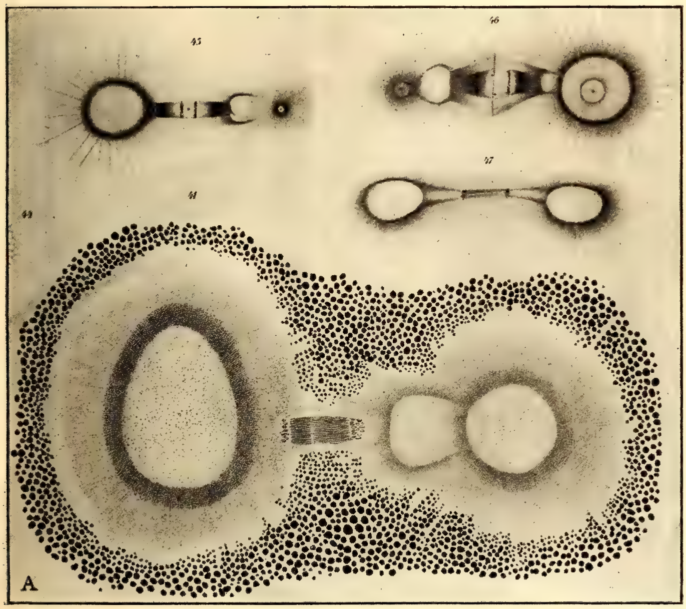

45
46
47
41
44
A

FIG. 4.

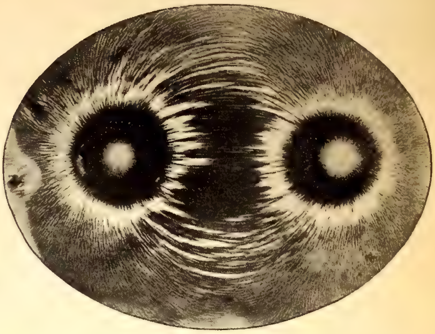


FIG. 6.

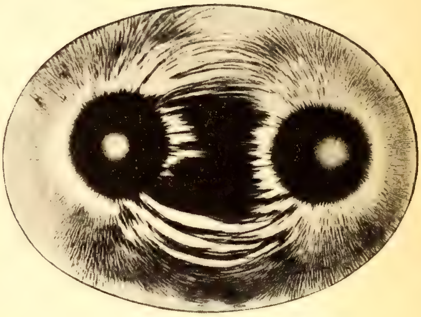


FIG. 7.

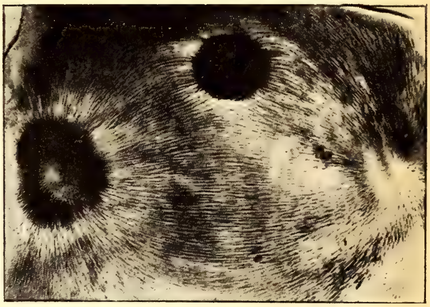


FIG. 8.

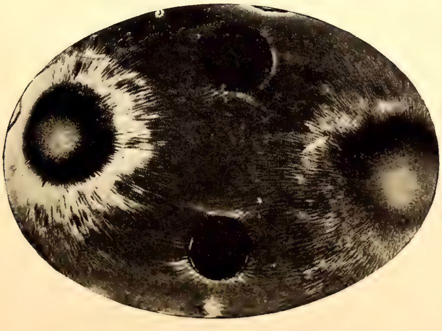


FIG. 9.

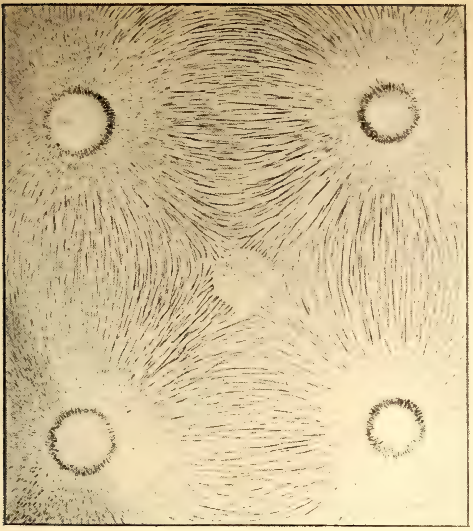


FIG. 10.

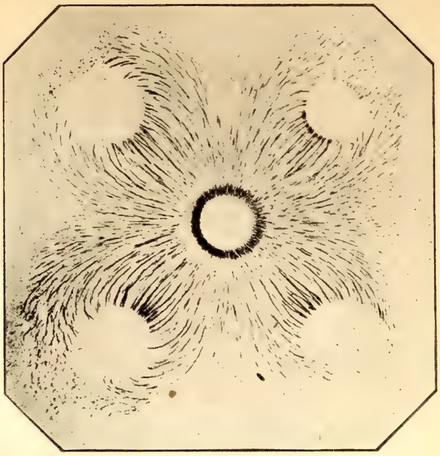


FIG. 11.

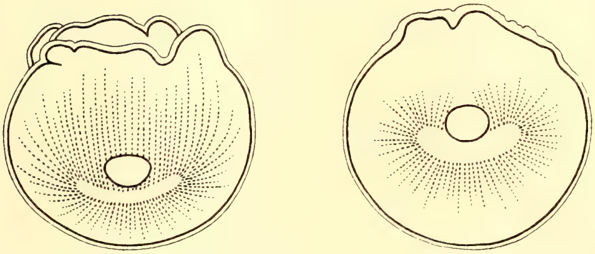


FIG. 12.

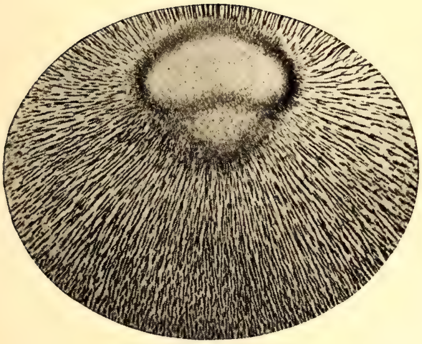


FIG. 13.

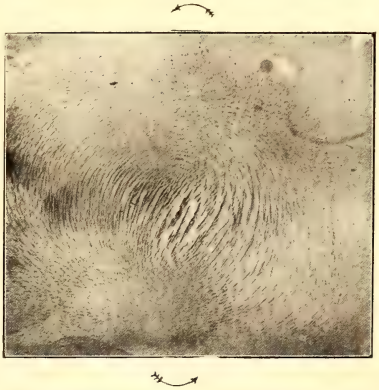


FIG. 14.

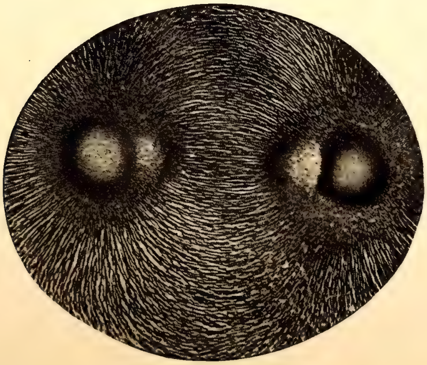


FIG. 15.

poles of a dual force. As the diffusion, osmosis, and surface-tension phenomena are of similar character at the two poles of a cell, they cannot be the forces involved in the spindle. Non-polarised undifferentiated forces, such as gravity, produce not the spindle-figure, but the "crossed field."

17. Since an isolated magnetic pole cannot exist (our model having simply served to show how the axial section of an *imaginary* field would behave), it is obvious that the cell-field, being in three dimensions, and with isolated unlike poles, cannot be due to magnetism.

18. It is not impossible that the field may be produced by statical electricity, but for the present there is no proof thereof.

EXPLANATION OF PLATES 9—11.

(Glycerine) (jelly) means that the dust was suspended in glycerine or jelly respectively; (dust) that magnetic dust was sifted on to paper, and figure obtained by tapping (see p. 555).

Figs. 1 and 2.—(From Wilson, "The Cell, etc.," ed. 2, 1902, figs. 25 and 26); (A) resting cell, two centrioles in centrosome; (B) formation of spindle; (C, D, E) further evolution of dumb-bell figure, and disappearance of nuclear wall to free chromosomes; (F) stage of "equatorial plate"; (G, H) splitting of chromosomes and discession of sister-segments; (I, 1) division of cell and reconstitution of daughter-nuclei.

Fig. 3.—After Vejdowský and Mrázek, in 'Arch. Mikr. Anat.,' vol. 62, 1903; stages of oosperm of *Rhynchelmis* (fig. 3, *a*, *b*), approximation and fusion of male and female gametonuclei; two successive zones of radiate centroplasm showing outside centrospheres; (*c*, *d*) "segmentation" figures, stage of equatorial plate.

Fig. 4.—After same; stages of discession of sister-segments of chromosomes, the centrosomes showed "blobbed" conditions.

Fig. 5.—Microphotograph of same preparation as fig. 3, *c* (Zeiss Ap. 8 mm., × 400 reduced $\frac{1}{2}$), showing the dumb-bell figure among dark-stained yolk spherules.

Fig. 6.—(Glycerine.) Current reduced as soon as figure developed. Anastomoses frequent.

Fig. 7.—(Glycerine.) After fig. 6 was taken, the magnetising current was again increased; the lateral chains have drifted inwards and become denser, leaving "Bütschli's spaces" on either side; numerous interlacings, and good differentiation of asters and spindle.

Fig. 8.—Glycerine triaster of two opposite poles and core.

Fig. 9.—(Glycerine.) Tetraster; two poles and two cores alternating.

Fig. 10.—(Dust.) Pentaster; four consecutive unlike poles with core in centre and spindle connections everywhere equivalent to the summation of four triasters.

Fig. 11.—(Dust.) Tetraster with central mass, or pentaster of four "like" surrounding a central opposite pole; the spindles have been made clearer by sweeping away the intervening dust.

Fig. 12.—Unipolar figures, after Boveri, showing crescentic centrosome and circular nucleus.

Fig. 13.—(Dust.) Model of 21; a crescentic plate over pole representing the centrosome; a disk in front of its concavity, the nucleus.

Fig. 14.—(Jelly.) Torsion figures; the arrows show direction of torsion.

Fig. 15.—(Dust.) Model of blobbed centrosomes, with small iron discs in front of the poles (compare fig. 4).

On the Probable Existence of Emulsin in Yeast.

By THOMAS ANDERSON HENRY, D.Sc. (Lond.), Principal Assistant in the Scientific and Technical Department of the Imperial Institute, and S. J. M. AULD, Ph.D. (Würzburg), Assistant in the Scientific and Technical Department of the Imperial Institute.

(Communicated by Professor Wyndham Dunstan, F.R.S. Received July 22, 1905.)

The observations of Treub,* Greshoff,† Jouck,‡ and other investigators have established the fact that hydrocyanic acid is furnished by a comparatively large number of plants belonging to a wide range of natural orders. Dunstan and Henry have applied to this process the name "cyanogenesis," and have shown that in many plants the production of hydrocyanic acid is due to the interaction of a cyanogenetic glucoside with a specific enzyme. Thus in *Lotus arabicus*, the hydrocyanic acid is produced by the decomposition of the glucoside lotusin by the enzyme lotase,§ in *Sorghum vulgare* by the action of emulsin on the glucoside dhurrin,|| and in the seeds of *Phaseolus lunatus* as the result of the decomposition of phaseolunatin by the enzyme emulsin.¶ The same authors have indicated that similar actions probably take place in cassava (*Manihot utilissima*), *Lotus australis* and *Chailletia cymosa*, all of which have been found to yield hydrocyanic acid when crushed in presence of water.**

The isolation of these cyanogenetic glucosides is often a matter of considerable difficulty, because, as a rule, they are only soluble in water and aqueous alcohol, and it is therefore a troublesome operation to separate them from the associated sugar (usually dextrose) and pectous matter which are also, in general, only soluble in the same solvents. In a few cases it has been found possible to remove dextrose from such mixtures by the action of phenylhydrazine, but this process usually leads to the loss of a portion of the glucoside, owing to partial condensation with the reagent.†† Some cyanogenetic glucosides are also slightly soluble in ethyl acetate, and this solvent

---

* 'Annales du Jardin Botanique de Buitenzorg,' vol. 9, p. 259.

† 'Berichte,' 1890, vol. 23, p. 3548.

‡ 'Inaug. Dissertat.,' Strassburg, 1902.

§ 'Phil. Trans., B, 1901, vol. 194, p. 518.

|| *Ibid.*, A, 1902, p. 399.

¶ 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

** 'Phil. Trans.,' A, 1902, vol. 199, p. 399, and 'Bulletin of the Imperial Institute,' 1903, vol. 1, pp. 12 and 112.

†† Dunstan and Henry, 'Phil. Trans.,' A, 1902, vol. 199, p. 402.

has been employed for the isolation of mandelonitrile glucoside,* and of dhurrin and phaseolunatin.†

With a view to devising a general process for the isolation of cyanogenetic glucosides we have, at Professor Dunstan's suggestion, investigated more thoroughly the properties of some of the known glucosides of this type, and the present paper contains an account of a number of results obtained in attempting to remove dextrose from mixtures of this sugar with cyanogenetic glucosides, by fermentation with yeast.

Action of Yeast on Amygdalin.

As a preliminary experiment, a solution of a mixture of equal quantities of amygdalin and dextrose in water was mixed with a small quantity of ordinary pressed yeast and allowed to stand in a warm place. Fermentation took place, carbon dioxide was evolved, and after several days a distinct odour of oil of bitter almonds was observed. This unexpected decomposition of the glucoside rendered necessary confirmatory experiments with amygdalin alone.

Amygdalin (2 grammes) was dissolved in 100 c.c. of water and about 6 grammes of ordinary pressed yeast added, together with a few drops of toluene to render the mixture antiseptic. The experimental flask was plugged with cotton wool and kept, together with a control flask containing a solution of amygdalin in water without yeast, at 40°. In the flask to which yeast had been added, the odour of benzaldehyde was observed after three days. This rapidly increased in intensity. After standing for two days more the mixture was distilled until free from hydrocyanic acid and the distillate, previously rendered slightly alkaline with potash, was extracted with ether. The oily residue left on distilling off the solvent had a strong odour of benzaldehyde, and the presence of this substance was proved by its conversion into dibenzylideneacetone (melting point, 112°), by condensation with acetone in presence of potash, and into benzaldehyde phenylhydrazone by the action of phenylhydrazine.

The aqueous solution left after extraction with ether was diluted to a known volume with water, and a portion examined for prussic acid with positive results. An aliquot part of the whole was then titrated with standard silver nitrate solution by Liebig's method, and by this means it was found that 33 per cent. of the amygdalin originally present in the solution had been decomposed by the yeast. The control flask was similarly examined, and it was found that in it no decomposition had occurred. A second experiment carried out on similar lines with another portion of the same specimen of yeast showed that after 11 days 67 per cent. of the amygdalin had been decomposed, after which the action appeared to cease. The residue left in the flask after distilling off the benzaldehyde and hydrocyanic acid was evaporated to dryness on the water-bath and extracted with alcohol. This solution was decolorised with animal charcoal and the solvent distilled off, leaving a quantity of unchanged amygdalin. This was extracted with ether. On evaporation of the solvent a minute quantity of a crystalline substance which was not amygdalin was obtained. The quantity of this material isolated was so small that it was impossible to further examine it, and its formation was not observed in any further experiment. It is possible that this substance was benzoic acid, since Herzog has shown* that salicyl alcohol is oxidised to salicylic acid by yeast, and it is conceivable that some of the benzaldehyde formed in this case may have similarly been oxidised to benzoic acid. No benzoic acid was obtained, however, by the action of yeast on small quantities of benzaldehyde suspended in water.

---

* Fischer, 'Berichte,' 1895, vol. 28, p. 1509.

† Dunstan and Henry, *loc. cit.*

The residue contained no dextrose, this having been changed by the zymase of the yeast forming alcohol and carbon dioxide.

This decomposition of amygdalin by yeast does not seem to have been observed previously, though Bourquelot has ascribed such an action to *Aspergillus niger*† and Gerard to *Penicillium glaucum*.‡

These experiments having established the fact that the hydrolysis of the amygdalin was brought about by the yeast, it was next sought to determine to which constituent of the yeast this hydrolytic action was due.

Fischer§ has shown that a preparation of invertase obtained by washing yeast with water has the power of partially hydrolysing amygdalin, forming one molecule of dextrose and one molecule of mandelonitrile glucoside according to the equation

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}O_{11}N} + H_2O = \underset{\text{Mandelonitrile glucoside.}}{C_{14}H_{17}O_6N} + \underset{\text{Dextrose.}}{C_6H_{12}O_6}.$$

This experiment was repeated and no difficulty was experienced in obtaining mandelonitrile glucoside by this means, and under no circumstances was any formation of hydrocyanic acid or benzaldehyde observed when such yeast washings were added to a solution of amygdalin. The complete hydrolysis of this glucoside by yeast cannot therefore be due to the action of invertase.

---

* 'Zeit. Physiol. Chem.,' 1903, vol. 37, p. 396.

† 'Compt. Rend. Soc. Biol.,' 1893, pp. 653 and 804.

‡ *Ibid.*, 1893, p. 651.

§ 'Berichte,' 1894, vol. 27, p. 2989; 1895, vol. 28, p. 1809.

The washed yeast so prepared had, moreover, lost none of its activity, and the following experiments, showing the rate at which *washed* yeast decomposes amygdalin, may be quoted as illustrative of this. For each experiment 2 grammes of amygdalin were dissolved in 50 c.c. of water contained in a small flask, and about 5 grammes of washed yeast were added to the solution. A number of flasks were prepared and kept at 40° in a water bath. At intervals a flask was taken out and the hydrocyanic acid formed estimated. The odour of benzaldehyde was first observed after the flasks had been kept at 40° for 74 hours.

Time after commencement of action.	Amount of hydrocyanic acid formed.	Amygdalin decomposed.
hours.	gramme.	Per cent.
2	0·006	5·5
24	0·012	10·5
48	0·016	14·0
72	0·019	16·0
120	0·038	31·8
168	0·052	42·0
240	0·085	70·8

The last observation probably represents the limit of decomposition, since another flask examined 48 hours later gave practically the same result. It will be observed that this limit is a little higher than that (67·8 per cent.) obtained with the unwashed yeast. A number of similar experiments were also made with the "Zymin" or "Acetondauerhefe," described by Albert, Buchner, and Rapp,* which is prepared by digesting brewers' yeast with acetone until the cell walls are ruptured and the contents egested. This material was also found to decompose amygdalin, though much less rapidly than pressed yeast. Thus, in one experiment 2 grammes of "Zymin" were added to an aqueous solution of 1 gramme of amygdalin and the mixture kept at about 40°; the odour of benzaldehyde was first observed after 74 hours, and at the end of 90 hours the hydrocyanic acid formed was estimated. This amounted to 0·0057 gramme, corresponding to 8·7 per cent. of the glucoside. A second experiment gave 0·0059 gramme of acid, equivalent to 9·8 per cent. of amygdalin, after 114 hours.

Experiments with Yeast Juice.

The action of yeast juice (Buchner's zymase)† on amygdalin was also investigated. For a liberal supply of this material prepared from fresh brewers' yeast by a slight modification of Buchner's process we are indebted to Dr. A. Harden of the Lister Institute of Preventive Medicine, to whom we take this opportunity of expressing our thanks.

---

* 'Berichte,' 1902, vol. 35. p. 2376.

† *Ibid.*, 1897, vol. 30, p. 117.

In the experiments with yeast juice on amygdalin, the flasks were plugged with cotton wool, their contents having been previously rendered antiseptic by the addition of a few drops of toluene. The temperature used was that found experimentally to be the best, viz., 40°.

Three different preparations of yeast juice were employed, and these, as the following table shows, varied considerably in activity.

Yeast juice.	Weight of amygdalin used in 20 c.c. of water.	Amount of yeast juice added.	Time required for commencement of decomposition.
	gramme.	c.c.	hours.
Specimen No. 1	1	5	23
" No. 2	1	5	36
" No. 3	1	5	120

Dilution of the juice had no very striking effect on the rate of decomposition, but the addition of a little water seemed to slightly accelerate the activity, and further addition of water to slightly diminish it, as the following table shows.

Weight of amygdalin used.	Volume of yeast juice.	Volume of water used.	Time required for commencement of decomposition.
gramme.	c.c.	c.c.	hours.
1	5	5	22·5
1	5	10	21
1	5	15	21·5
1	5	20	23
1	5	25	24

The products of the action of yeast juice on amygdalin are the same as those of yeast itself, viz., benzaldehyde, hydrocyanic acid, alcohol and carbon dioxide.

Influence of Hydrocyanic Acid on the Glucosidolytic Action of Yeast Juice.

That hydrocyanic acid of moderate strength has comparatively little disturbing action on the glucosidolytic activity of yeast is obvious from the fact that the decomposition of amygdalin can proceed to the extent of 70 per cent. of the amount of glucoside used. It will be seen from the following table that hydrocyanic acid exerts very little, if any, inhibiting action on the glucosidolytic activity of yeast juice, even in comparatively strong solutions. On the contrary Buchner has shown* that this acid lessens the activity of the zymase in yeast juice.

Weight of amygdalin used.	Volume of yeast juice added.	Volume of 1 per cent. solution of hydrocyanic acid added.	Length of time during which action proceeded.	Amygdalin decomposed.
gramme.	c.c.	c.c.	hours.	Per cent.
1	5	—	36	12
1	5	5	36	11·5
1	5	10	36	12
1	5	20	36	11

It is well known that the activity of the zymase of yeast juice decreases when the juice is kept,† and this is generally ascribed to the action of the proteolytic enzyme (endotryptase) first detected in yeast by Hahn and Geret.‡ It was consequently considered advisable to ascertain whether the glucosidolytic action of yeast juice similarly diminishes on keeping. The juice was kept at the atmospheric temperature and its activity towards amygdalin tried at intervals.

Juice.	Time of standing.	Time required to initiate the decomposition of amygdalin.
	hours.	hours.
Specimen No. 1 ……	24	22
" No. 1 ……	47	23
" No. 1 ……	120	26
" No. 2 ……	24	40
" No. 2 ……	120	56

It is evident from these results that the glucosidolytic activity undergoes slight diminution when the yeast juice is kept, but this diminution is in no way comparable with that which the activity of the zymase undergoes under the same conditions. For purposes of comparison, some experiments were made to ascertain whether the commoner proteolytic enzymes interfered to any extent with the action of the emulsin of almonds. For this purpose 1 gramme of Merck's emulsin was thoroughly mixed with 100 c.c. of water, and to measured portions of the mixture a weighed quantity of commercial preparations of pepsin, trypsin, and papain were added. After a certain period had elapsed, portions of these mixtures were allowed to act on solutions of amygdalin, and the amounts of hydrocyanic acid formed after definite periods were determined.

---

* 'Berichte,' 1898, vol. 31, p. 2672.

† Albert and Buchner, 'Berichte,' 1900, vol. 33, p. 971.

‡ 'Berichte,' 1898, vol. 31, p. 202.

As a control experiment, 20 c.c. of the emulsin mixture were allowed to stand, and at intervals 2 c.c. were added to 1 gramme of amygdalin dissolved in 20 c.c. of water, and the amount of the glucoside decomposed estimated after a certain lapse of time.

Control Experiments with Emulsin.

Time allowed to stand.	Time of action on amygdalin.	Amygdalin decomposed.
hours.	hours.	Per cent.
—	18	65·7
25	18	63·2
50	18	56·8

Experiments with Emulsin and Proteolytic Enzymes.

Proteolytic enzyme.	Weight of proteolytic enzyme.	Volume of emulsin solution.	Time of proteolytic action.	Time of action of emulsin on amygdalin.	Amygdalin decomposed.
	gramme.	c.c.	hours.	hours.	Per cent.
Pepsin	0·2	3	26	24	None
		3	50	[2—3 days]	„
	0·2	5	25	24	2·0
		5	50	48	None
	0·2	20	24	18	32·0
		20	50	24	8·7
		20	106	24	None
Trypsin	0·2	3	26	24	47·3
		3	50	24	33·0
	0·2	5	25	24	51·3
		5	50	24	38·0
Papain	0·2	5	24	18	60·7
		5	50	24	58·3

These results show that, of the three proteolytic ferments used, pepsin exerts a powerful destructive action on the activity of the emulsin of almonds, trypsin a well-marked action, whilst papain affects it slightly. It will be observed that the slight action exerted by trypsin on emulsin is similar to that of the proteolytic enzyme of yeast on the glucosidolytic constituent of yeast, and this is in conformity with Kutscher's observation* that the proteolytic enzyme of yeast resembles trypsin in its action. (*Compare also Hahn and Geret.**)

---

* 'Zeit. Physiol. Chem.,' 1901, vol. 32, p. 59.

Effect of Antiseptics on the Glucosidolytic Action of Yeast.

The activity of the glucosidolytic constituent of the yeast is not affected by antiseptics, and in this respect, as also in its behaviour towards dilute mineral acids and alkalis, by which its action is totally inhibited, it resembles the emulsin of almonds.

The experiments recorded in the following table were carried out under comparable conditions at 40°. In each case 1 gramme of amygdalin was used, and the hydrocyanic acid formed was estimated by Liebig's method.

Substance.	Amount of antiseptic.	Weight of yeast used.	Time.	Hydrocyanic acid formed.	Amygdalin decomposed.
		grammes.	hours.	gramme.	Per cent.
(Control)	—	3	90	0·0063	10·5
Chloroform	1 c.c.	3	90	0·0060	10·0
Toluene............	1 ,,	3	90	0·0061	9·8
Phenol	0·02 gramme	3	90	0·0060	10·0

Action of Yeast on Glucosides.

The results of the experiments already described indicate that the action of yeast on amygdalin is probably due to the presence in the yeast-cells of an enzyme of the type of emulsin, and it was considered desirable to ascertain whether or not the glucosidolytic constituent could be definitely identified with this enzyme.

The identification of an enzyme is at present a somewhat difficult problem, since it is as yet impossible to isolate bodies of this type in a pure state. Recourse must therefore be had to the investigation of the specific action of the ferment, and especially to the range of temperature over which it is active, the nature of the substances it decomposes, and the characters of the decomposition products. For this purpose the action of yeast on a number of glucosides other than amygdalin was examined. The principal results obtained were as follows:—

Salicin.—Yeast decomposes salicin in precisely the same manner as emulsin, forming saligenin. The latter was isolated, and identified by means of its melting point (82°), and its characteristic colour reaction with ferric chloride. It might have been expected that some salicylic acid would have been formed, due to the further action of the yeast on the saligenin,† but no trace of this acid could be detected. The dextrose first formed in this action is changed by the zymase of the yeast forming alcohol and carbon dioxide.

---

* 'Zeit. Biol.,' 1900, vol. 40, p. 117.

† Compare Herzog, *loc. cit.*

Mandelonitrile Glucoside.—Fischer has shown that this glucoside is readily hydrolysed by emulsin. It is also hydrolysed by yeast; thus, when yeast was added to a solution of 0·5 gramme of the glucoside dissolved in 10 c.c. of water and the mixture kept at 40°, the odour of benzaldehyde was observed after 36 hours, and after 72 hours 40 per cent. of the glucoside present had been decomposed. The dextrose first formed was decomposed by the zymase of the yeast.

Other Glucosides.—In the same way it was found that arbutin and phaseolunatin were decomposed by yeast, whilst quercitrin, digitalin and sinalbin were unattacked by it.

It seemed possible that this action of yeast on glucosides might be due to the direct fermentation of the sugar residues present in these substances by the zymase contained in the yeast or its preparations, with the result that the molecule underwent total disruption, the products other than sugars being non-fermentable and, therefore, remaining intact. This explanation of the action is, however, not permissible in view of the fact that the action of yeast is restricted to certain types of glucosides, and that it does not decompose digitalin or quercitrin, although these contain residues of the fermentable sugars, digitalose and rhamnose respectively. Moreover, Fischer has asserted that disaccharides are never fermented directly by yeast, and that the latter only attacks hexoses produced by the preliminary decomposition of disaccharides by hydrolytic enzymes such as invertase, maltase and lactase, and bearing in mind the analogy in constitution between glucosides and the disaccharides established by Fischer, it is probable that the same rule holds good with regard to glucosides.

Fractionation of Yeast Juice by Heat Coagulation.

Wroblewski* has shown that when yeast juice is heated, coagulations of proteid matters occur at certain definite temperatures, and that in particular the filtrate from the coagulate produced at 41° is practically free from zymase.† Wroblewski's experiments were repeated, small tubes containing the yeast juice being heated gradually to various temperatures between 40° and 70°. The coagulates obtained at these various temperatures were filtered off and the activity of the filtrate in each case towards amygdalin and dextrose was determined. The principal coagulates were found to be produced at 48°, 55°,

---

* 'Berichte,' 1898, vol. 31, p. 3218, and 'Journ. prakt. Chem.,' 1901, vol. 64, p. 1.

† Compare Buchner, 'Berichte,' 1899, vol. 32, p. 2086.

58°, and 65°. These temperatures differ somewhat from those given by Wroblewski, being on the whole a few degrees higher, but agreement in this respect is scarcely to be expected in experiments of this kind, since the formation of the various precipitates is probably due to the coagulation in turn of different proteid matters, including the enzymes, and the temperatures at which the coagulates are formed probably depends to some extent on the concentration of the various proteids in the yeast juice, and these, in turn, will vary with the previous history of the yeast from which the juice is prepared. The results obtained in one set of these experiments are shown in the following table:—

Temperature to which juice was heated.	Time of heating.	Change observed.	Activity of filtrate towards—	
			Glucose.	Amygdalin.
degrees.	minutes.			
41	90	None	Active	Active
42	20	None		
46	18	Turbidity		
48	25	Voluminous precipitate...	Slight	Benzaldehyde formed after 47 hours
50	20	None	None	Active
53	40	Slight flocculence		
55	30	Voluminous precipitate...	...	Benzaldehyde formed after 45 hours
56	20	None		
58	30	Voluminous precipitate...	...	Benzaldehyde formed after 34 hours
63	15	Turbidity		
66	25	Voluminous precipitate...	...	Benzaldehyde formed after 58 hours
70	20	None	...	Benzaldehyde formed after several days
70—72	20	None	...	No action

It was observed in all the sets of heat coagulation experiments that the activity of the yeast juice towards amygdalin increased as each of the first few coagulates was removed. This increase in activity is no doubt due in part to the gradual concentration of the glucosidolytic enzyme in the liquid as the result of continued heating, but it may also be due in part to the removal of other enzymes, especially the endotryptase which, as has been shown, appears to gradually destroy the glucosidolytic enzyme. The maximum activity of the juice towards amygdalin is reached when it has been heated at 58° (according to Hahn and Geret* the endotryptase is destroyed when the yeast juice is heated at 60°), further heating diminishes the activity, which finally disappears at about 70°. Yeast juice which has been heated to 70° still hydrolyses sucrose and must, therefore, contain invertase.

---

* *Loc. cit.*

Fractional Precipitation of Yeast Juice with Alcohol.

Attempts were made to isolate a definitely active preparation of the enzyme by treating yeast juice with successive small quantities of alcohol and collecting the several precipitates so produced. It was found, however, that these precipitates exhibited, in a less degree, all the activities characteristic of yeast juice itself. Recourse was, therefore, had to the alcoholic precipitation of yeast juice which had been heated previously to 58° and subsequently filtered. The precipitate so obtained was washed with dilute alcohol, spread on glass, and dried by exposure over desiccating agents under reduced pressure. This preparation contained the glucosidolytic enzyme and invertase, but was free from endotryptase and zymase.

As in all the previous experiments in which the action of yeast or yeast preparations on amygdalin was investigated, no dextrose could be found among the hydrolytic products, this having been decomposed by the zymase, it was thought worth while to investigate more fully the action of this new preparation on amygdalin. About 0·1 gramme was added to 20 c.c. of a 2-per-cent. solution of amygdalin in water and the mixture maintained at 40°. The odour of benzaldehyde became noticeable after 90 hours. The action was allowed to proceed for some time and then the benzaldehyde was extracted with ether and identified by conversion into dibenzylideneacetone. The presence of hydrocyanic acid among the hydrolytic products was proved by the application of the usual tests. To the liquid, left after removal of benzaldehyde, phenylhydrazine acetate was added and the mixture warmed at 100°. After about 20 minutes the phenylosazone which had separated was collected, washed, and recrystallised from alcohol. It melted at 205°. A specimen of phenylglucosazone prepared at the same time melted at 205°.

Identification of the Glucosidolytic Enzyme of Yeast.

The data afforded by the results of the experiments already described are that the glucosidolytic enzyme of yeast hydrolyses amygdalin, salicin, arbutin, phaseolunatin, and mandelonitrile glucoside, but does not attack sinalbin, digitalin, or quercitrin. The temperature at which its activity is destroyed is about 70°, and it is most active at 40°. Its activity is inhibited by the presence of small quantities of alkalis or acids, but not by antiseptic agents.

A comparatively large number of glucosidolytic enzymes have been described, but of those which hydrolyse glucosides containing the —CN group or —CNS group only the following are known :—

Emulsin, which decomposes amygdalin, dhurrin, phaseolunatin and gynocardin.

Lotase, which hydrolyses lotusin and appears to be otherwise inactive.

Gynocardase, which hydrolyses gynocardin.

Myrosin, which hydrolyses sinigrin (potassium myronate) and sinalbin and appears to be otherwise inactive.

Of these four enzymes it is possible that gynocardase may prove to be identical with emulsin,* and assuming this, it is evident that the activity of each of the three remaining enzymes is associated with a particular type of glucoside; thus lotase reacts with lotusin, which differs from all the known cyanogenetic glucosides in having the —CN group attached to the sugar residue; myrosin reacts only with glucosides having the —CNS group attached to the non-sugar portion of the molecule, and emulsin decomposes glucosides having the —CN group associated with the non-sugar portion of the molecule. Power and Gornall have, however, obtained from the seeds of *Taraktogenos Kurzii,* a preparation which is stated to decompose both potassium myronate and amygdalin.† This may be due to the simultaneous presence in this preparation of two ferments, one of the emulsin and the other of the myrosin type.

It will be seen that the range of activity of the glucosidolytic enzyme of yeast coincides with that of emulsin in so far as the nature of the glucosides decomposed is concerned. As regards the influence of change of temperature on the activity of emulsin scarcely any observations are on record, and it was considered worth while to ascertain whether, like the yeast enzyme, emulsin ceases to be active at about 70°. It is difficult to secure strictly comparable conditions for such experiments, since there is no known means whereby equivalent solutions of the two materials can be procured; thus, in some comparative experiments, it was found that 1 c.c. of a liquid obtained by shaking up 1 gramme of Merck's emulsin with 100 c.c. of water when added to 10 c.c. of a 10-per-cent. solution of amygdalin in water decomposed 85 per cent. of the glucoside in 20 hours, whilst 5 grammes of yeast, under the same conditions, decomposed only 6·5 per cent. of the amygdalin in the same time, whence it would appear that if the glucosidolytic activity of yeast is due to the presence of emulsin, the specimen of yeast used in this instance could have contained only 0·0001 of that contained in the specimen of commercial emulsin used.

It was, however, satisfactorily established that a remarkable diminution in the activity of emulsin can be brought about by heating, and that total disappearance of activity takes place at about the same temperature as that observed in the case of yeast. The results recorded in the following table were obtained with a liquid containing 1 gramme of Merck's emulsin thoroughly mixed with 100 c.c. of water:—

---

* Power and Lees, 'Journn. Chem. Soc.,' 1905, vol. 87, pp. 354 and 357.

† 'Journ. Chem. Soc.,' 1904, vol. 83, p. 841.

Temperature to which liquid was heated.	Time of heating.	Time required for decomposition of amygdalin by 1 c.c. of the liquid after heating.
degrees.	hours.	minutes.
64	2	30
65	1	30
66	1	40
68	1	40
69	1	90
70	75 minutes	150
71	30 "	30 hours
71	45 "	no action

These results show that although the temperature at which emulsin becomes inactive is practically identical with that at which the activity of the glucosidolytic enzyme of yeast ceases, emulsin may be exposed to the same temperatures as yeast juice for a much longer time before its glucosidolytic activity is destroyed. This is no doubt due to the fact that the concentration of the emulsin in the preparation used in this set of experiments was much greater than in the yeast juice used in the experiments described on p. 577.

It has been shown, therefore, that the glucosides which are decomposed by yeast are those which are attacked by emulsin, and further that the conditions under which these decompositions are effected by yeast, especially as regards temperature, are those which are operative in the case of emulsin. Taking all these facts into consideration there seems to be little room for doubt that the glucosidolytic activity of yeast is due to the secretion of emulsin in the cells of the plant.

On the Inheritance of Heterostylism in Primula.

By W. Bateson, M.A., F.R.S., and R. P. Gregory, M.A.

(Received July 31, 1905.)

In view of the results obtained by Darwin,* Hildebrand and others, it seemed likely that the characters long-style and short-style, well known in Primulaceæ and other orders, might have a Mendelian inheritance. Our experiments have shown that this is the case in *P. sinensis,* the short style being dominant, the long recessive.

The inheritance is usually of the simplest type. In one case (p. 584) there was considerable divergence from the expected proportions, and it is no doubt possible that this case was one of real abnormality; but we incline to think that the irregularity was due to accident or error. But besides the cases which can be regarded as normal one individual short-styled plant gave an entirely aberrant result (p. 584); and as the offspring of this plant gave results similarly aberrant, there can be little question that we are here concerned with an inheritance of a special type. Further experiments with this family are in progress.

Another feature of interest was seen in the F_2 families raised from matings in which an *equal*-styled race was used, the phenomena well illustrating the mode of appearance of a new type by the recombination of the factors brought in by the pure parental types.

Horticultural experience as to the production of long- and short-styled offspring is in general harmony with our results. Fashion has decreed that *P. sinensis* shall be exhibited in the long-styled form alone. This being the recessive, breeds true, and short-styled plants are consequently absent from selected strains, being even difficult to procure at the present time. The florists' Auricula, on the contrary, must be exhibited in the short-styled or "thrum" form, but as this is the dominant, long-styled Auriculas continue abundant.

In the wild Primrose (*P. acaulis,* Jacq.) the two forms are about equally numerous in nature. Experiments with this species, now in progress, give indications that the inheritance of the two types follows the same rules. From the greater sterility of its illegitimate unions the Primrose is less easy to work with, and as might be expected from the same cause, all short-styled wild plants so far tested, have been found to be heterozygous in respect of style.

---

* 'Forms of Flowers,' edit. 1884, giving references to the principal memoirs on the subject.

The experiments now to be described all relate to *P. sinensis.* The inheritance of flower-colour and other characters will be dealt with in a future communication. We take this opportunity of acknowledging our indebtedness to Messrs. Sutton and Sons, who have for some years placed their great collection of Primulas at our disposal, and have assisted us in many ways during the course of our inquiries.

I.—NORMAL CASES.

Long-styled × *Long-styled.*

Ten such crosses were made, from which 90 offspring were raised, all long-styled. In F_2 56 offspring, all long, were raised. Various extracted long-styled recessives, fertilised by self, and by pure longs, gave 85 plants, all long.*

Short-styled × *Short-styled.*

All the four short-styled plants originally obtained for use in these experiments proved to be heterozygous. From short-styled × short-styled, 26 short and 10 long were raised, the expectation being 3 : 1. Of the 26 short some were DD and others DR. One, on self-fertilisation, gave seven shorts. Two others, on self-fertilisation, gave 24 short, four long. Nine shorts raised in F_1 from long × short gave, on self-fertilisation, 120 shorts and 49 longs. The union DR × DR gave, therefore, a total of 144 short, 53 long; or, including the 36 raised between the original plants, 170 short, 63 long.

Darwin's† results from this form of union are valuable as indicating that he probably obtained a pure dominant. The parent short-styled plant, self-fertilised, gave eight plants, seven short, one long. The shorts, self-fertilised, gave only two plants which flowered (short), but the cross between short-styled and long-styled gave 15, all short.

Long ♀ × *Short* ♂, *and Reciprocal Cross.*

In the first instance these crosses were all R × DR or DR × R, which gave respectively 30 short, 24 long; and 14 short, 13 long. In F_2, crosses between DD and R gave 92 all short; and DR × R gave 40 short, 48 long.

Crosses with an Equal-styled Race.

Of late years a peculiar type of *P. sinensis* has been much grown, which is characterised by an extensive spreading of the central yellow eye. Instead of forming a fairly sharp pentagon as in normal flowers, the eye in this type is produced as a yellow flush extending over about half of each petal. All the strains having this flush are in the condition called by Darwin "equal-styled." The anthers are at the same level as in the long-styled flowers, and the pollen grains are small and indistinguishable from those of the long-styled. The styles, however, are short and do not reach above the level of the anthers.* We at first supposed that the equal-styled plants corresponded to the mid-styled type seen in trimorphic species, but this is evidently a mistake, and the relations of the three types of trimorphic forms present much greater complexity than is met with in Primula.

---

* *Cf.* Darwin, *loc. cit.*, pp. 213, 214.

† *Loc. cit.*, p. 215.

Experiment shows that the yellow flush is an ordinary recessive character, the ordinary or non-flushed type being dominant. The flush is transmitted independently of the length of style or the size of the pollen grains, for it may be transferred to the true short-styled or "thrum" type. But when the flush is developed in plants which by gametic composition would be long-styled, the style does not pass through the anthers, and the equal-styled condition is produced. Why the development of the yellow flush in these flowers should entail the reduction of the style, we cannot in any way suggest.

From these considerations it follows that when the equal-styled race is crossed with the true short-styled type, two allelomorphic pairs are concerned, viz., short-style (D) and long-style (R); no yellow flush (D) and yellow flush (R). F_1 is, therefore, short-styled with no yellow flush. F_2 has four types, viz., short, non-flushed; short, flushed; long, non-flushed; long, flushed, which latter is the equal-styled, the ratio being 9 : 3 : 3 : 1. The long non-flushed, which appears as a new form in F_2, is, of course, made by the recombination of the parental characters, and the meeting of the "long" character from the equal-styled parent with the non-flushed eye derived from the short-styled parent.

Equal-styled × *Equal-styled.*

Four plants were raised by crossing equal-styled plants of the same race, and did not differ from their parents. From these were raised 14 more by self-fertilisation, again identical with their parents.

---

* Occasional flowers, in which the stigma is at the anther-level, may be seen on normal long-styled plants. They are usually first flowers, and are especially frequent in *P. acaulis* in early spring. We have never seen a genuine case of mixture of types on one plant.

Equal-styled × Long-styled.

The yellow flush being recessive, F_1 is here the normal non-flushed long style; 45 such plants were first raised, all long and without the flush. In the next year, 77 such plants were produced by similar matings.

Such F_1 plants gave by self-fertilisation 183 long, non-flushed, 51 equal-styled, with the flush, the expectation being 3 : 1. Crossed with the pure recessive, they gave 93 long, non-flushed, 107 equal-styled, with the flush, numerical equality being expected.

Equal-styled × Short-styled.

From such crosses, in which the short-styled parents were DR, 39 plants were raised, 19 long, 20 short, all without yellow flush. The pure short-styled plants raised in 1903, crossed with pure equal-styled plants, gave 41, all short-styled, without flush.

Such F_1 plants on self-fertilisation gave 247 plants, viz., 147 short, non-flushed, 35 short, flushed, 44 long, without flush, 21 equal flushed, the expectation being 138·9, 46·3, 46·3, 15·4.

Crossed with ordinary longs, the same F_1 plants gave 73 short, 76 long, all without flush, the expectation being equality.

The same F_1 plants, crossed with the pure equal-styled, gave 59 short, non-flushed, 39 short, flushed, 32 long, non-flushed, 24 long, flushed. This result, showing in each class a great excess of shorts, instead of numerical equality, is quite unexplained. The numbers can scarcely be taken as chance departures from equality. The same plants, however, gave recognisably normal results in both their other sets of matings, and the segregation was evidently quite normal. On the whole, it seems more likely that the aberration was due to accident, than that any novel phenomenon actually occurred in this case.

II.—ABNORMAL CASES.

With the exception just mentioned, all the cases hitherto dealt with gave fairly simple Mendelian results, but the entire series of crosses in which a certain short-styled plant (referred to as No. 6) was used showed a definite and consistent departure from normal expectation. No. 6 was a red thrum plant, obtained from a nurseryman, and we know nothing of its origin. By self-fertilisation it gave four shorts. Fertilised by a short-styled plant, which had been proved to be DR, it gave six shorts, three longs. No. 6 was used as male on both long- and equal-styled plants, giving 10, all short-styled; but when fertilised with pollen of long- and equal-styled plants, No. 6 gave 14 short, 5 long.

The evidence so far is, therefore, that the *pollen* of No. 6 gave a mixture of longs and shorts, and consequently was carrying both characters, while all the plants raised from it as *female* were shorts. The numbers alone are of course too few to justify any conclusion, had it not been that a closely similar result appeared in the next generation.

By self-fertilisation No. 6 gave a short-styled plant, No. 37. This, on self-fertilisation, gave 22 shorts and no longs. Fertilised by pollen of long-styled plants, it gave 14 short, 24 long. But when used as a male parent, its pollen applied to long- and equal-styled plants gave 148 shorts and only 4 longs, of which one was recorded as "doubtful."*

Taking their offspring together, Nos. 6 and 37, when fertilised by long- and equal-styled, gave 29 long, 28 short; while when the same two plants were used as *males*, the total offspring were 184 shorts and 4 (? 3) longs. We have, therefore, the remarkable phenomenon of plants which, judged by the female gametes, were ordinary heterozygotes, while their male gametes were almost exclusively bearing the dominant character. Pending further investigation, we can offer no further comment on this singular case. It will be noted that, since the mixture was given by the *female* side, no hypothesis of parthenogenesis will meet the case.

Results of Double Pollination.

In addition to the experiments described above, an attempt was made to investigate another possibility respecting the consequences of legitimate and illegitimate unions. Darwin, and after him many others, proved that in Primula more seeds are produced when plants with styles of dissimilar types are united (legitimately) than when similars are united (illegitimately). Nevertheless, illegitimate unions are not necessarily sterile, but, especially in the case of *P. sinensis,* may produce a good deal of seed.

For examples we may refer to the average numbers given by Darwin.† Taking the average for legitimate unions at 100, the 13 illegitimate unions in the genus *Primula* give an average of 53 seeds per capsule, and we have found a similar proportion maintained with some constancy in our own fertilisations.

Some egg-cells (about half) are therefore fertilised by illegitimate pollen, while the rest are not. This fact suggested that there may be a differentiation between egg-cells of the same plant, such that some are capable of illegitimate fertilisation, others incapable.

---

* The nature of the doubt is not recorded. Until the results were added and classified no special interest had been attached to this family. Each plant as it began to flower was recorded and thrown away to make room. Probably this individual was recorded before the flower completely matured.

† *Loc. cit.*, p. 246.

To test this possibility, we made a large number of trials with *P. acaulis* and *sinensis,* pollinating some flowers legitimately, some illegitimately, and others with pollen of both types. We anticipated that the double pollinations, in which pollen of both types was put on the same stigma, would produce a maximum number of seeds. In the case of *P. sinensis,* by making use of the fact that green stem and pinnate leaf are recessive to red stem and palmate leaf, it was possible to arrange these double pollinations in such a way that the paternity of each resulting seedling would be apparent, and thus the number of individuals derived from each set of pollen grains could be ascertained.

This series of experiments has, however, led to no definite conclusion. They were carried out through two seasons, and an enormous number of fertilisations were made, but the resulting figures were so discrepant that we are unable to give either a positive or a negative answer to the question proposed. These discrepancies are partly due to great individual differences between plants and between flowers of the same plant, but in all probability serious irregularities were also introduced in the actual operations owing to the difficulty of applying the two sorts of pollen equally to the same stigma under really uniform conditions. If these technical difficulties could be overcome, a valuable result might possibly follow from the experiment.

Further Experiments and Histological Investigations on Intumescences, with some Observations on Nuclear Division in Pathological Tissues.

By Miss ELIZABETH DALE.

(Communicated by Professor H. Marshall Ward, F.R.S. Received March 18,—Read April 6, 1905.)

(Abstract.)

This paper is the third of a series on intumescences.

The first was mainly anatomical, the second chiefly experimental, and both related to one species, viz., *Hibiscus vitifolius.* The present paper contains (1) an account of further experiments with different plants, chiefly with *Solanum tuberosum* and *Populus tremula.* On the potato plant intumescences were obtained experimentally in about 24 hours, either on a complete and uninjured plant, or on single leaves, or on small fragments of leaves. As in the case of *Hibiscus vitifolius,* the effect of varying degrees of temperature and illumination were investigated, and also the influence of nutritive solutions on floating leaves—the constant factor being a saturated atmosphere. A close connection was established on experimental, developmental, and cytological grounds between intumescences and wound-callus.

(2) Additional anatomical observations were made, and a classification of various types of intumescences, based on development and anatomical characters, has been drawn up. The cell contents of intumescences on various plants have been examined and compared with a view to discovering the osmotic substance which causes the initial accumulation of water in the formation of intumescences.

(3) In this connection experiments were made to determine whether the relative amounts of acids and salts, respectively, differed in plants with intumescences as compared with healthy individuals.

(4) These observations and experiments, taken in connection with various theoretical considerations, led to the conclusion that the actively osmotic substance is an acid, probably *oxalic acid.* They further, as do the experiments with fragments of leaves, show conclusively that the internal causes of the formation of intumescences are *extremely local,* and that root pressure is in no way concerned with the process.

The results obtained confirm and extend the conclusions reached by the earlier work with *Hibiscus vitifolius,* viz., that *moist air, heat, light,* and

generally, oxygen are the necessary external factors. But the more recent investigations bring out the importance of certain internal or biological factors, namely, *irritability* and either *active powers of assimilation* or abundance of stored food material.

(5) Finally, the nuclear phenomena were investigated and compared, and were found to be in every respect identical in various intumescences and in wound-callus. Pathological tissues in certain plants and animals are also compared, and a strong resemblance is seen to exist between certain *rapidly* formed outgrowths in plants and animals, caused not by any parasitic organism, but simply by the influence of some stimulus, probably always external, acting upon a plant or animal in such a condition of irritability that it is able to respond. A similar resemblance occurs between regenerative wound tissues in certain plants and animals, the formation of which is in all cases accompanied exclusively by the more rapid form of nuclear division known as amitotic or direct.

The Experimental Treatment of Trypanosomiasis in Animals.

By H. Wolferstan Thomas, M.D., C.M. (McGill), (J. H. Todd Memorial Fellow in Tropical Medicine), Liverpool School of Tropical Medicine, Johnston Tropical Laboratory, University, Liverpool.

(Communicated by Professor R. Boyce, F.R.S. Received April 8,—Read May 11, 1905.)

Numerous drugs have been tried in endeavouring to find a therapeutical agent which would cause trypanosomes to disappear from the blood. Of all those tried, arsenic and trypan red are the only ones exhibiting a marked influence on the parasites.

On account of the liability of the appearance of toxic symptoms, the proneness to sloughing at the site of inoculation, and the oftentimes considerable disturbance which occurred after the subcutaneous administration of sodium arseniate, I decided to try if other compounds would be more satisfactory in the treatment of trypanosomiasis. The arsenic preparation which has given the best results is a compound of arsenic and aniline $C_6H_5No_2As$ (meta-arsensaure anilid, atoxyl), a preparation which has of recent years been used intravenously and subcutaneously in the treatment of skin diseases and anæmia.

The effect of this preparation in the treatment of trypanosomiasis has been observed in an extensive series of experiments during the last 10 months.

Strains used.

T. gambiense.—Five strains. A very virulent one obtained from one of my cases of Sleeping Sickness—this strain exhibited nothing abnormal in its direct passage through a monkey, but after infecting a baboon (*Cynocephalus babuin*), and then passing into a rabbit, it became abnormally virulent.

T. evansi.—Surra. *T. equinum.*—Mal de Caderas. *T. brucei.*—Nagana. *T. equiperdum.*—Dourine. *T. dimorphon.*—Gambian horse.

No animal was treated until a large number of parasites were observed in the blood and definite signs of anæmia and loss of weight determined. Many of the animals were not treated until several months after infection had occurred.

Four methods of treatment were adopted—

(*a*) Atoxyl—high doses at intervals of a week.

(*b*) Atoxyl—high initial dose and then reduced amounts administered three times a week.

(*c*) Atoxyl and trypan red combined.
The arsenic preparation given first and then followed in 36 to 72 hours with high doses of the dye subcutaneously.

(*d*) Trypan red alone.

All of these methods of treatment were continued over a period of several weeks to three months, or until a decided improvement in the general condition of the animal was noticed, especially increase of weight, rise in hæmoglobin and number of erythrocytes, with absence from the blood of the parasite.

In nearly all cases controls were used. In every case the treated animals have survived their controls. From time to time blood was taken from the treated animals and susceptible animals were inoculated. After a varying length of time treatment was discontinued, and some of these animals, after a period of one to three months without treatment, were bled to death, and the whole blood injected into healthy animals. Such control animals have remained uninfected.

RESULTS.

Treatments A and B.

T. gambiense.—Rabbits, guinea-pigs, and rats after one and a-half to three months' treatment have survived. Treatment discontinued four to five months ago.

T. evansi.—One rabbit, two guinea-pigs are alive three months after stopping treatment. It is now seven months since the guinea-pigs were infected.

T. brucei.—Four guinea-pigs, three months after treatment, were bled, and rats inoculated with whole heart blood have remained uninfected during one and a-quarter months. Rabbit two months treated, one month later still well. Twenty rats have survived four months. One rat infected on fifth day of disease when parasites were present, 150 or more to a field, living, and blood negative 84 days later.

T. equinum.—One rabbit, treatment begun only when characteristic discharge from eyes, nose, etc., appeared—treatment for two months—discontinued one month, animal apparently well. Two guinea-pigs have survived three months without treatment. Rats, so far, 101 days.

T. equiperdum.—Pups, one has died from over dose, other negative and general condition better.

T. dimorphon.—Results not so good. Animals have lived a far longer time than controls, but no apparent cure can be recorded.

Treatment C.

With this method animals require treatment for a shorter period, but many have died from toxic effects of the dye; this is especially the case with dogs, pups, and kittens.

T. dimorphon.—Results of combined treatment far more favourable. Experiments in progress. Trypan red alone.

Results with *T. equinum,* rats have lived 197 days.

Effects on animals infected with *T. gambiense, T. evansi, T. brucei, T. dimorphon, T. equiperdum,* are in accordance with Laveran's experiments. No definite curative powers remarked.

Experiments with the sodium arseniate treatment of animals infected with the various trypanosomes have given less favourable results. Greater tendency is noted for toxic symptoms and sloughing to occur. High doses cannot be tolerated for a prolonged period. *In my hands the arsenic-aniline compound has given far better results than treatment with sodium arseniate. The advantages of its administration intravenously or subcutaneously in high doses over a length of time, namely, its less toxic properties, the absence of all tendency to cause sloughing, and the apparently longer action of the drug, make me believe that the employment of this compound is indicated in the treatment of human trypanosomiasis.*

The combination with trypan red is worth trying in small doses per os, but the appearance of any indication of nephritis or other toxic symptoms should cause the treatment to be immediately stopped. I have administered in pill-form six to eight grains three times a week for a period of three and a-half weeks without any untoward symptoms. The case was a native suffering from trypanosomiasis; the parasites lessened in number, and by the end of the third week were hardly ever seen.

Ehrlich and Shiga found that they could protect animals if trypan red was administered (either subcutaneously or per os). After five to seven days such animals could be injected with virulent blood containing trypanosomes without infection occurring.

I have injected a goat with increasing doses of trypan red, and, after a time, used its serum. Mice infected with the Mal de Caderas parasite were injected with small quantities of this serum. In four cases the animals lived 31 to 48 days. Experiments in progress.

Studies on Enzyme Action. VII.—*The Synthetic Action of Acids contrasted with that of Enzymes. Synthesis of Maltose and Isomaltose.*

By Edward Frankland Armstrong, Ph.D., D.Sc., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College.

(Communicated by Professor H. E. Armstrong, F.R.S. Received July 29, 1905.)

[*International Catalogue of Scientific Literature.*

Author's title slip :—D. Q.
Subject slips :—
D 1810 Glucose, condensation of.
D 1820 Maltose and Isomaltose. Synthesis of
D 7090 Condensation of Glucose.
D 8012 Enzymes. Synthetic action of
Q 1230 Enzymes. Synthetic action of
Q 1416 Maltose and Isomaltose. Synthesis of, by Acids and Enzymes.]

The belief has grown up of late years that the enzymes which are capable of inducing the hydrolysis of disaccharides or bioses act reversibly ; as yet, however, but little has been done to define the theory of the process and no understanding has been arrived at as to the limitations to which such changes are subject. The same is true of the action of acids, which also act reversibly under certain conditions.

Croft Hill,* whose observations gave rise to the conception of reversible enzyme action, at first thought that maltose alone was produced by the action of the enzyme maltase on glucose. Emmerling,† who repeated Croft Hill's experiments, came to the conclusion that the product was isomaltose, the biose which E. Fischer‡ obtained by subjecting glucose to the action of concentrated chlorhydric acid. In a later communication,§ while still claiming that maltose is formed in small quantity, Croft Hill has admitted that the chief product is an isomeride of maltose; but he regards this as different from isomaltose and therefore terms it *revertose*.

By subjecting a mixture of galactose and glucose such as is obtained by hydrolysing milk sugar with lactase to the action of this enzyme, a disaccharide, isolactose,‖ is formed, which is undoubtedly isomeric with milk

* 'Chem. Soc. Trans.,' 1898, p. 634 ; 'Ber.,' 1901, vol. 34, p. 1380.

† 'Ber.,' 1901, vol. 34, pp. 600 and 2206.

‡ 'Ber.,' 1890, vol. 23, p. 3687 ; 1895, vol. 28, p. 3024.

§ 'Chem. Soc. Trans.,' 1903, p. 578.

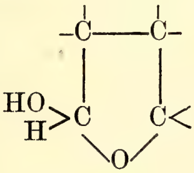
‖ E. Fischer and E. F. Armstrong, 'Ber.,' 1902, vol. 35, p. 3151.

sugar, as the synthetic product is completely fermented by bottom yeast, a yeast having no action on milk sugar; moreover, as the solution has no reducing action after the removal of this product by fermentation, the presence of milk sugar is precluded.

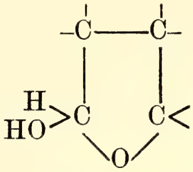
This observation is scarcely compatible with the statement made by Croft Hill in his latest communication that, whilst a large number of isomeric bioses are conceivably possible, "one would expect that with any particular enzyme or group of enzymes only those would be formed which are capable of being hydrolysed back to glucose by the same enzyme:" the very opposite would appear to be the case, as will be shown later on.

If the argument made use of in previous communications be correct, the action of acids and of enzymes, both as hydrolytic and as condensing agents, is similar, except that, and in so far as, differences arise owing to the non-selective activity of the former and the strictly selective activity exercised by the latter.

The key to the interpretation of the changes which attend condensation must be looked for in the behaviour of glucose itself in solution. It is now abundantly proved that glucose can exist in two stereoisomeric forms, differing only in the position which the hydroxyl group occupies relatively to the oxygen atom in the ring, viz.:—



α-Glucose.*



β-Glucose.

The term glucose, in fact, has a double connotation and these two substances must usually be thought of under the single name. As crystallised from alcohol, it consists almost entirely of the α-form; but this changes over into the β-form if maintained during several days at about 105°.† If either form be dissolved in water, change takes place of the one into the other: ultimately, the two forms exist in solution in equilibrium, in proportions which depend on the conditions, the β-compound predominating.‡

---

* The two positions are labelled arbitrarily.

† *Cf.* Tanret, 'Bull. Soc. Chim.,' 1905, vol. 33, p. 337.

‡ Tanret, who was the first to recognise that glucose existed in several forms, has been led by the results recorded in No. 1 of this series to reconsider the conclusion he originally came to that three isomerides are obtainable. He now agrees that there are but two, corresponding to the two α- and β-methylglucosides, and that his supposed third modification was an equilibrated mixture of these two forms. Calculating from the rotatory power ($[\alpha]_D = +110°$) of the pure α- and $[\alpha]_D = +19°$ of the pure β-form, he

Change takes place in a similar manner in other media. If the glucose be dissolved in methylic alcohol containing hydrogen chloride, it undergoes etherification, each of the two glucoses being converted into the corresponding methylglucoside:* only one of these (the α-compound) is hydrolysed by maltase; the other (the β-compound) is hydrolysed by emulsin. The behaviour of these two glucosides is typical of that of glucosides generally, which are divisible into two groups, the α and the β, according as they are hydrolysed either by maltase or by emulsin. It is noteworthy that, apart from the sugars proper, in all cases in which the test can be applied, the natural glucosides have been found to belong to the β-group.

The process by which a monose is converted into a biose must be regarded as precisely similar to that by which α-glucose and β-glucose are converted into the two methylglucosides: the behaviour of maltose, in fact, is such as to characterise it unquestionably as glucose-α-glucoside; isomaltose is presumably the stereoisomeric glucose-β-glucoside.

When glucose undergoes condensation "uncontrolled," it should give rise to both maltose and isomaltose, the proportions of which ultimately present in equilibrium would depend on their relative stability under the conditions operative at the time. But, inasmuch as hydrolysis under the influence of enzymes is an absolutely selective process, being so controlled that it takes place in one direction only, it might be supposed that synthesis under their influence would also be a controlled operation and that the tendency of the enzyme would be to reproduce the biose which it hydrolyses: apparently this point of view was present in Croft Hill's mind and led him to suppose, at first, that maltose was the actual product; as a matter of fact, it is uncertain at present whether maltose is produced at all: it is certainly not the sole nor even the predominant product.

The formation under the influence of the enzyme of a single biose, *isomeric with that which it hydrolyses,* could be accounted for on the assumption that both are produced initially but that the one again undergoes hydrolysis as soon as it is formed, so that it all but disappears. If, however, it were shown that only the stereoisomeride of the biose hydrolysed is produced initially, it would be necessary to regard the synthetic activity of the enzyme as opposed to its hydrolytic activity. An explanation which in a measure unites both

---

infers that the proportion in which these are present in equilibrium is $a = 37$ per cent., $\beta = 63$ per cent. in a 10-per-cent. solution, and $a = 40$, $\beta = 60$ in a concentrated solution.

Lowry ('Trans. Chem. Soc.,' 1904, p. 1551), who bases his conclusions on determinations of solubility, takes the view that a solution of glucose contains a considerable proportion of glucose aldehydrol in addition to a- and β-glucose; but his argument cannot be regarded as a convincing one ('Comp. Jungius. Zeit. Phys. Chem.,' 1905, p. 103).

* E. F. Armstrong and S. L. Courtauld, 'Proc. Physiol. Soc.,' July, 1905.

points of view is that the enzyme acts throughout by "protecting" one or the other position, according as it belongs either to the α- or to the β-class of hydrolysts, thereby practically preventing condensation from taking place in more than one direction; in other words, assuming that it be the function of the enzyme to bring water into the circuit of change at the precise spot where it is required to effect the hydrolysis of a biose, it might serve, when acting on the products of hydrolysis, to maintain water in such a position as to hinder the condensation from occurring in the direction which would involve the reversal of the operation of hydrolysis; condensation would then be confined to the alternative position and would give rise to the biose which is the correlative of that hydrolysed by the enzyme.

There can be no doubt that the enzyme has a specific influence in promoting the formation of the biose which it cannot hydrolyse, as no action takes place in its absence or when the solution is heated sufficiently to destroy it. To understand the character of this influence, it should be remembered that α- and β-methylglucoside have both been shown to be capable of entering into close association with maltase and with emulsin; presumably, therefore, the two forms of glucose present in solution both combine with the enzyme. Inasmuch as the enzymes are capable of acting as hydrolytic agents, they must, like acids, be capable also of acting as dehydrating agents. Probably the two forms of glucose give rise to different results, because, while hydrolytic action prevails in the one system, in the other the dehydrating effect is alone exercised, the association of the α-enzyme with α-glucose being of such a nature that water is continually present and can be made use of at the centre where the condensation should take place, the dehydrating effect being therefore almost entirely in abeyance; whilst in the enzyme-β-glucose system the configuration of the enzyme relatively to the β-glucose is such as to render hydrolysis impossible and consequently the dehydrating effect prevails. In both cases opportunity conditions action: the different actions are begotten of different opportunities.

It is noteworthy that, under natural conditions, apparently only one product is formed, there being no evidence, for example, that isomaltose is ever present in the plant. If further investigation should render this conclusion absolute, it will follow that the control exercised under natural conditions is not merely that which the separated enzyme exercises. But attention must not be confined to the enzyme, as the argument used above tends to show that, even under the influence of maltase, maltose alone might be produced, if the conditions prevailing in the plant be such as to give rise only to α-glucose, provided that the maltose were immediately withdrawn from the sphere of action, for example, by diffusion and fixation as starch;

and that such may be the case is by no means improbable, as it is conceivable that if glucose were formed against a maltase template,* it would be present initially only in the α-form.

It will be obvious from these statements that it is all-important to determine the nature of the product, both when condensation is effected by ordinary chemical means and also when effected by means of an enzyme.

1. Proof is given in the present communication that when the condensation is effected under laboratory conditions the action takes place in the manner indicated above; in other words, the two products required by theory are both formed.

2. Evidence is adduced to show that isomaltose is the β-glucoside correlative with the α-glucoside maltose.

3. Experiments are described bearing on the formation of isomaltose by the agency of the α-enzyme maltase and of its correlative maltose by the agency of the β-enzyme emulsin which leave little doubt that the two bioses are producible from glucose.

4. And whilst it is left undecided whether maltase can give rise to maltose, evidence is cited which at least renders it probable that emulsin does not give rise to isomaltose.

Synthesis of Maltose and Isomaltose by means of Chlorhydric Acid.

To effect the condensation of glucose, E. Fischer used ordinary chlorhydric acid. The precipitate obtained on mixing the liquid with a large quantity of alcohol and ether was dissolved in water and all fermentable matter was removed from the neutralised solution by means of brewers' yeast. Such a method of purification would have destroyed any maltose which had been formed. Fischer does not appear, however, to have contemplated the formation of this sugar.

In my experiments, a stronger acid was used and this was afterwards removed by means of lead carbonate. Appropriate yeasts were used to destroy one or the other carbohydrate.

One hundred grammes of glucose having been dissolved in 300 c.c. of concentrated chlorhydric acid, the mixture was cooled to 0° and hydrogen chloride gas was passed in until the colour commenced to darken. The liquid was kept below +10° C. during about 40 hours, when the temperature was allowed to rise to 15°. After neutralising the acid by stirring the liquid with lead carbonate, the filtrate and washings were shaken with silver carbonate to remove the dissolved lead chloride.

Finally an almost colourless neutral solution was obtained. Judging from its behaviour with phenylhydrazine, this contained a biose, together with much glucose. The manner in which isomaltose and maltose were detected will be apparent from the following descriptions:—

---

* 'Roy. Soc. Proc.,' 1904, vol. 73, p. 538.

Proof of the Presence of Isomaltose.—To remove the unchanged glucose, about 20 c.c. of boiled yeast-water were added to the liquid, which was then sterilised and inoculated with a quick-acting pure yeast, *S. intermedians*, Hansen. After fermentation had gone on during 10 days at 25°, the solution was filtered, mixed with a little calcium carbonate to neutralise any acid which had been formed during the fermentation, boiled to expel alcohol and then reinoculated with yeast under sterile conditions. These operations were usually repeated a second time, experience having shown such repetition to be the only way in which the last trace of fermentable sugar can be removed.

The solution finally obtained, when clarified with charcoal, was almost colourless; the total volume was 150 c.c.; its rotatory power in a 1-decimetre tube was $\alpha_D = 4^\circ{\cdot}20$.

When a mixture of about 15—20 c.c. of the solution with 2—3 c.c. of almost colourless phenylhydrazine, dissolved in 3 c.c. of 50 per cent. acetic acid, was heated in a flask in a boiling water bath during 1—1½ hours, no separation of osazone took place from the hot liquid, which was an indication that no glucosazone was formed. On cooling the liquid, a light yellow flocculent osazone separated out slowly; this was filtered off, well washed with cold water and redissolved in boiling water, in which it was entirely and easily soluble. The osazone only crystallised out when the solution was nearly cold, differing in this respect from maltosazone, which crystallises out while the solution is still hot. After a second crystallisation from water, the osazone was crystallised from dilute alcohol; it then formed a light brownish yellow crystalline powder, which decomposed when heated at about 120°. To complete the purification, it was next crystallised from wet ethylic acetate. The slightly yellow micro-crystalline powder thus obtained was very soluble in boiling water; on heating, it melted at about 156°, forming a brown liquid, which decomposed at 198°, behaving in every way exactly in the manner described by E. Fischer.

It was to be supposed that if it were a derivative of glucose, it would be hydrolysed by emulsin: 30 c.c. of the solution were therefore mixed with a small quantity of active emulsin and a few drops of toluene; the liquid was then set aside in a closed flask during several days at 38°, along with a similar quantity of the solution to which no enzyme had been added. To test whether glucose had been formed, the filtered solutions were heated side by side with phenylhydrazine. In the one case, separation of an insoluble glucosazone was observed after 20 minutes' heating; in the control experiment no separation took place but a soluble osazone was formed.

Proof of the Presence of Maltose.—In order to destroy the glucose, the liquid was fermented with *S. Marxianus,* a yeast which does not contain maltase and therefore is without action on maltose. After removal of the fermentable matter, the solution, 200 c.c. in volume, containing the product from 50 grammes of maltose, had the rotatory power $\alpha_D = +7^{\circ}\cdot35$, which was about double that observed in a similar solution containing isomaltose alone. The osazone formed on heating with phenylhydrazine was entirely soluble in boiling water, but distinctly less soluble than the isomaltosazone; it began to separate from the liquid while this was still hot and the osazone could be fractionated into more and less soluble portions.

Separate portions of the solution were digested with emulsin and maltase; in both cases glucoses were formed. When subjected to the joint action of emulsin and a yeast containing maltase (*S. intermedians*), the solid matter in solution disappeared almost entirely in a single fermentation.

The isomaltose is apparently present in larger quantity than maltose: it is proposed to determine their relative proportions by removing the isomaltose from the mixture by the joint action of emulsin and *S. Marxianus.*

The Production of Isomaltose by means of Maltase.

The maltase extract used was always prepared by grinding 5 grammes of air-dried top yeast (from a London brewery) with 100 cm. of water and then digesting the mixture at 25° during two to three hours. Fifty grammes of glucose were dissolved in about 75 c.c. of the filtered extract, and some toluene was added to maintain the liquid sterile; the solution was kept in a stoppered bottle at 25° during two to three months or even longer; it darkened somewhat but remained perfectly clear and sweet. To remove glucose and maltose, the solution diluted with an equal volume of water was boiled with charcoal and filtered; yeast water was then added and after the liquid had been sterilised it was fermented with *S. intermedians.*

To insure complete removal of the glucose, it was necessary to repeat the fermentation at least twice. Ultimately, a clear solution was obtained which not only had a strong reducing action on Fehling's solution but also a high positive rotatory power. When treated with phenylhydrazine in the manner described, it gave an osazone in every way identical with isomaltosazone; in fact, when the experiments were carried out side by side with the products obtained by means of acid and by means of the enzyme, no difference could be detected. The product also behaved in the manner to be expected towards emulsin, being converted into glucose.

The Production of Maltose by means of Emulsin.

A solution of 50 grammes glucose in 75 c.c. water was mixed with 1 gramme of emulsin. The liquid was kept at 25° during about two months: then filtered, diluted and freed from glucose by continued fermentation with *S. Marxianus* in the manner described.

Ultimately, a solution was obtained which contained a disaccharide yielding a phenylosazone soluble in boiling water but separating quickly on cooling; the product crystallised in plates, which, when recrystallised from wet ethylic acetate, melted and decomposed at 200°, which is but very few degrees below the melting point of pure maltosazone.

The matter in solution underwent almost complete fermentation when inocculated with a yeast containing maltase (*S. intermedians*); only traces of reducing sugar remained, showing that little, if any, isomaltose could have been formed.

These observations leave practically no doubt that the substance present was maltose. The yield under the conditions hitherto observed has been very small.

The experiments will be continued with the object of obtaining both maltose and isomaltose in a separate crystalline state.

Studies on Enzyme Action. VIII.—*The Mechanism of Fermentation.*

By Edward Frankland Armstrong, Ph.D., D.Sc., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College.

(Communicated by Professor H. E. Armstrong, F.R.S. Received July 29, 1905.)

[*International Catalogue of Scientific Literature.*

Author's title slip :—D. Q. R.

Subject slips :—

D 1800 Fermentation of carbohydrates by various yeasts.
D 7090 Fermentation. Mechanism of alcoholic
D 8020 Alcoholic fermentation. Mechanism of
Q 0235 Yeasts, various, action of, on carbohydrates.
Q 1240 Fermentation of sugars by various yeasts. Mechanism of
R 1820 Yeasts, various, action of, on carbohydrates.]

In view of the suggestion which has been made* that the mechanism of fermentation is, perhaps, to be correlated with that whereby the enzymes effect hydrolysis, it is of importance to ascertain, if possible, the manner in which the activity of the various organisms giving rise to alcoholic fermentation is dependent on or influenced by the enzymes which they contain. The correlation of the activity of an organism with that of its constituent enzymes is of special significance also on the biological side, in connection with the inquiry into the possibility of alterations in the activity of organisms being brought about by changes in their environment.

The only systematic investigation made in this direction is that carried out by E. Fischer, who studied the behaviour of most of the known sugars towards a dozen different "pure" yeasts, cultivated from single cells, which (excepting *S. Marxianus* and a milk sugar yeast) all contained both invertase and maltase. The conclusion arrived at was that only the four hexoses: glucose, mannose, fructose and galactose are fermentable.

A systematic search through the literature of the subject—mostly to be found in bacteriological and brewing journals—shows that a large number of isolated observations have been made on the fermentative power of individual yeasts; as a rule, however, the experiments made by various observers, if not incomplete, have been conducted under more or less different conditions.

Two points stand out prominently: without exception, yeasts which ferment glucose also ferment fructose and mannose in all cases in which their action on these sugars had been tried; quite a number of yeasts are described as being without action on galactose. The evidence available on these two points was sufficient to make it very important to extend the inquiry under uniform conditions, to determine the action of each of the yeasts used on the four hexose sugars above named, as well as to ascertain the nature of the enzymes present in each of the yeasts.

---

* 'Roy. Soc. Proc.,' 1904, vol. 73, 541.

The points borne in mind primarily throughout the investigation were:—

1. Does every yeast ferment glucose, mannose and fructose equally well?
2. Do all yeasts ferment galactose?

The experiments were begun in the Carlsberg Laboratory, at Copenhagen, which I visited specially for the purpose last summer; I desire to take this opportunity of expressing my indebtedness to Professor E. C. Hansen, both for the interest he displayed in the work and for the generosity with which the resources of his laboratory were placed at my disposal. I have also to thank Dr. Schionning for the manner in which he aided me to master the methods of cultivating and using pure yeasts so successfully elaborated in the Carlsberg Laboratory.

It may fairly be claimed that the yeasts selected represent as wide a range of variation in morphological characters and other respects as possible; conclusions based on their behaviour can, therefore, be applied in any discussion of the behaviour of the saccharomyces generally.

The following is a list of the yeasts used:—*

Saccharomyces cerevisiæ.
„ *Carlsberg.*
„ *Thermantitonum.*
Schizosaccharomyces Pombe.
Saccharomyces Marxianus.
„ *exiguus.*
Saccharomycodes Ludwigii.
Willia anomala.
„ *Saturnus.*
Schizosaccharomyces octosporus.
Saccharomycopsis capsularis.
„ Klocker yeast.
Saccharomyces fragilis.
Torula Kayser.
„ *Adametz.*
Kefir yeast.
Yeast No. 698.
Saccharomyces apiculatus.
„ „ *Schweiz.*
„ *mali Duclauxi.*

A few words as to the morphological characteristics of these yeasts may be of interest. *S. cerevisiæ* is a typical English top-fermentation yeast, *S. Carlsberg* is a typical low-temperature bottom-fermentation yeast; *S. Pombe,* originally obtained from Africa, exercises its maximum activity at 36°, forming much alcohol; *S. Thermantitonum,* a yeast recently described by

---

* For the nomenclature adopted, compare Hansen, 'Centralblatt für Bakteriologie,' 1904, vol. 12, p. 529.

Grove Johnson,* is remarkable on account of its ability to withstand a temperature as high as 83° C., and as being most active at 45°; *S. exiguus* differs from *S. Marxianus* in that the latter forms a membranous film; *S. anomalus* and *S. Saturnus* are characterised by the occurrence of both acids and fruit ethers among the products of their fermentative activity. The other yeasts are characterised by the manner in which they undergo reproduction, as their group names show: the torulæ do not form spores; the schizosaccharomyces multiply by fission; *S. capsularis* forms spores with two enveloping membranes; and *S. apiculatus*, which is very common on fruits in the late summer and autumn, occurs in very characteristic, small, lemon-shaped cells.

The method adopted was as follows: The yeasts, which in nearly all cases had been obtained from the Carlsberg Laboratory, were cultivated in beer wort; subcultures consisting of quite young cells not more than 24 hours old were always used in the actual experiments. The sugars were highly purified† and were used in the form of 10-per-cent. solutions in yeast water. A single drop of the yeast sediment was introduced into about 10 c.c. of the sterilised sugar solution contained in a Freudenreich flask, with the rigid precautions prescribed by Hansen to ensure sterility. The solutions were maintained at 25° while under observation. Up to the present time, the experiments have been made solely with the object of ascertaining whether or no the organisms were able to bring about the fermentation of the various sugars.

In positive cases, the evidence of the occurrence of fermentation afforded by the rapid evolution of small bubbles of gas, especially on shaking, was unmistakable. Sometimes only a very few bubbles were observed to escape: such a result was regarded either as indicating the presence of traces of impurity or as due to the fermentation of glycogen in the yeast. In most cases the production of alcohol was confirmed by distillation. As a rule, the occurrence of fermentation was evident within 24 hours and was very marked within 48 hours.

The present communication is intended to be only a preliminary note; it is proposed to discuss the full bearing of the results in a subsequent communication in connection with those of other workers and the theories of fermentation.

---

* 'Journ. Inst. Brewing,' 1905, vol. 11, p. 466.

† It may be well to point out here that many samples of galactose contain traces of glucose; in fact, to remove all traces of glucose from commercial galactose, it is necessary to subject it to the action of a yeast such as *S. Ludwigii*, which ferments glucose readily but is without action on galactose.

The results which have been obtained are summarised in the following table. The sign + is used to indicate that a particular sugar is fermented, the sign 0 to indicate that it is not fermented. Inasmuch as a biose must be hydrolysed before it can be fermented, the ability or the reverse of a yeast to ferment a given biose sugar denotes the presence or absence of the particular enzyme which will hydrolyse the biose; the signs in the three columns headed maltose, sucrose and lactose respectively may therefore be read as implying the occurrence or absence, as the case may be, of fermentation, as well as the occurrence or absence of a particular enzyme. Although inability to hydrolyse a biose when it has diffused into the yeast cell would seem to be as severe a test of the absence of the enzyme from the cell contents as it is possible to apply, it has been thought desirable in several cases to grow the yeast in quantity and to prepare an extract from the dried material; in all such cases the result has been that no enzyme has been proved to be present the absence of which was indicated by the behaviour of the yeast towards the bioses.

Yeast.	Glucose	Fructose	Mannose	Galactose	Maltose (maltase).	Sucrose (invertase).	Lactose (lactase).
Yeasts containing maltase and invertase.							
Cerevisiæ	+	+	+	+	+	+	0
Carlsberg	+	+	+	+	+	+	0
Pombe	+	+	+	0	+	+	0
Thermantitonum ...	+	+	+	+	+	+	0
Yeasts containing invertase alone.							
Marxianus	+	+	+	+	0	+	0
Exiguus	+	+	+	+	0	+	0
Ludwigii	+	+	+	0	0	+	0
Saturnus	+	+	+	0	0	+	0
Anomalus	+	+	+	0	0	+	0
Yeasts containing maltase alone.							
Octosporus	+	+	+	0	+	0	0
Capsularis	+	+	+	+	+	0	0
Klocker	+	+	+	0	+	0	0
Yeasts containing lactase and invertase.							
Fragilis	+	+	+	+	0	+	+
Kefir	+	+	+	0	0	+	+
Kayser	+	+	+	0	0	+	+
Adametz	+	+	+	0	0	+	+
698	+	+	+	+	0	+	+
Yeasts without sucroclastic enzymes.							
Apiculatus............	+	+	+	0	0	0	0
„ Schweiz	+	+	+	0	0	0	0
Mali Duclauxi* ...	+	+	+	?	0	0	0

* I have not worked with this yeast.

The results recorded in the table allow of a definite answer being given to the two questions propounded in the introduction. It will be noticed that, without exception, all the twenty typical yeasts tested were able to ferment glucose, mannose and fructose; and it should be added that they did so, apparently, with equal ease.

But quite a number of the yeasts were incapable of fermenting galactose, including even *S. Pombe*, which acts very powerfully on glucose. In this connection it may be mentioned that Dienert* has stated that of 89 yeasts examined by him, all of which fermented glucose, as many as 21 were unable to ferment galactose. As a rule, galactose was fermented less easily than the three other sugars; but in this connection it should be mentioned that Maze† has described several yeasts which, he states, ferment galactose more readily than glucose.

Whatever may be the mechanism of alcoholic fermentation, it is beyond doubt that inability to ferment galactose has nothing to do with the absence from the yeast of any one of the sucroclastic enzymes, since yeasts are to be found which are without action on galactose in each of the four classes. It must therefore be supposed that this sugar is not merely somewhat less readily fermented than the other three sugars but that the process is in itself a distinct one; that, in fact, the fermentation of glucose and galactose is brought about by different mechanisms.

The power of a yeast to ferment mannose or glucose or fructose is clearly in no way conditioned by the presence of a particular sucroclastic enzyme; indeed, taking the experiments with *S. apiculatus* into account, it would seem that the occurrence of alcoholic fermentation is altogether independent of the presence of an enzyme—whether free or fixed—able to induce the hydrolysis either of maltose or of sucrose. The same argument is applicable to the fermentation of galactose.

But although it must be concluded that the process of enzymo-hydrolysis and of fermentation are different in some essential respects, it is apparently none the less certain that they are cognate phenomena; otherwise it would be difficult to understand why it is that the only hexoses to undergo fermentation are those which have been shown to be compatible with the sucroclastic enzymes (compare No. III of these Studies). It is conceivable that the enzyme is potentially present, in a more or less modified form, in the protoplasmic structure of the cell, in a condition in which it can induce fermentation; and that it is only on liberation from the protoplasmic structure that it assumes sucroclastic functions, whilst, at the same time, it loses its fermentative activity.

---

* 'Ann. Inst. Pasteur,' 1900, p. 39.

† *Ibid.*, 1903, p. 11.

The fact that the three hexoses which behave alike have one common enolic form (compare No. III of these Studies) is of utmost significance as an indication that the formation of the enol is the initial stage in the fermentation of the hexose and that the breakdown of the molecule commences at the terminal carbon atom. This conclusion derives support from the fact that the methylglucosides, gluconic acid, ethylic gluconate and similar compounds—in which only the groups attached to the terminal carbon atom differ from those in glucose—are unfermentable.

The formation of the enol cannot take place without the carbon-oxygen ring undergoing rupture. It is in this direction perhaps that an explanation will be found of the fact that the bioses cannot be fermented directly or without previously undergoing resolution into the simpler hexoses. To judge from the behaviour of compounds such as the methylglucosides, it is probable that the conversion of the glucose into a glucoside involves an increase in the stability of the ring structure: perhaps, therefore, the ring structure in the bioses is of sufficient stability to remain unruptured in solution; in other words, it is possible that, whereas a solution of glucose contains the three isodynamic forms of the compound, viz., α-glucose, β-glucose and the corresponding enol—the last being present probably only in small proportion and as the hydrated compound or aldehydrol—a solution of a biose such as maltose contains only the two isodynamic forms corresponding to the two glucoses.

Studies on Enzyme Action.—Lipase.

By Henry E. Armstrong, F.R.S.

(Received July 29, 1905.)

[*International Catalogue of Scientific Literature.*
Author's title slip :—D. Q.
Subject slips :—
D 8014 Q 1240 Ricinus Lipase.]

It was first shown by J. Reynolds Green, in 1889, that germinating seeds of the castor-oil plant contain an enzyme which is capable of hydrolysing castor oil. Of late years an industry has been based on this discovery, as it has been found that if ground ungerminated castor-oil seed be mixed with an ordinary fatty oil and a small quantity of acid (preferably acetic), the fatty matter is almost entirely hydrolysed into glycerol and fatty acid: as the change takes place rapidly, at a little above an ordinary temperature, the hydrolysis of fats may be effected in this manner with considerable advantage on economic as well as other grounds.*

The study of vegetable lipase is of special importance, as the ordinary fats —which are hydrolysed under its influence with peculiar readiness—are not asymmetric material but simply glycerides of acids of the acetic or oleic series. The discovery of the nature of the process is, therefore, of particular interest, in order that a comparison may be instituted between this enzyme and those which are known to act selectively. The interest of the inquiry is enhanced by the fact that animal lipase, according to Dakin,† acts selectively on the mixture of ethereal salts derived from inactive mandelic acid, hydrolysing the dextro-constituent more rapidly than the lævo-constituent; but even in this case, inasmuch as the whole of the ethereal salt is hydrolysed eventually, the selective effect of lipase is of a different order from that displayed, for example, by an enzyme of the sucroclastic class, which can only attack one member of a pair of enantiomorphous isomerides.

Among the chief points of interest already established by Connstein and his fellow workers are, firstly, that Ricinus lipase is effective only in presence of acid; secondly, that it acts preferentially on the natural fats; other ethereal salts are scarcely if at all attacked by it.‡

---

* 'Comp. Connstein: V. Internationaler Congress für Angewandte Chemie,' Berlin, June, 1903, vol. 2, p. 537.

† 'Journal of Physiology,' 1903, vol. 30, p. 253; 1905, vol. 32, p. 199.

‡ The literature of the subject is summarised in an article by Connstein, "Ueber fermentative Fettspaltung," in Asher and Spiro's 'Ergebnisse der Physiologie, Biochemie,' 1904, vol. 3, p. 194.

Animal lipase, however, from the liver or pancreas of the pig, according to Kastle and Loevenhart,* manifests considerable activity in hydrolysing simple ethereal salts, the action being greater in the case of the higher than of the lower terms of the series, as shown by the fact that ethylic butyrate is more readily hydrolysed than is ethylic acetate. Dakin's observations may be regarded as confirming this conclusion. Whether animal lipase acts on natural fats is not yet satisfactorily determined. Lewkowitsch states that he could not carry the hydrolysis of cotton-seed oil beyond 3 per cent.; he is inclined to attribute this want of success, however, to the fact that he could not secure a satisfactory emulsion.

The activity of animal lipase, it should be mentioned, is said by Magnus† to be conditioned by two substances, one of which is dialysable and not destroyed by boiling whilst the other is destroyed by heat and not dialysable.

Judging from my own experiments, it is clear that the investigation of lipase presents peculiar difficulties. It would scarcely be worth while to put the results on record, were it not desirable to call attention to issues which they raise.

The material used was simply ground castor-oil seed, free from husk, when the action of the enzyme on castor oil was under consideration; whilst for the purpose of studying the action of the enzyme on other ethereal salts, this material was carefully freed from oil by washing it with ether and dried by exposure to the air on a porous plate. In nearly every case, toluene was added to maintain sterile conditions.

Connstein's contention has been confirmed that the presence of acid is necessary to condition the hydrolysis and that practically any acid is effective, provided a sufficient amount be used. Aspartic and glutamic acids—which are formed at an early stage of the germination of seeds—were found to be highly active; glycin and asparagin, however, were practically without effect. Thus, in an experiment in which a mixture of 5 c.c. of olive oil, 1 gramme fat-free castor-oil seed and 10 c.c. 3/100 N sulphuric acid was digested at 38° during 18 hours, the amount of oleic acid liberated was 4·145 grammes. In a blank experiment from which the enzyme was omitted, the amount of oleic acid liberated at the end of 18 hours was 0·1087 gramme; at the end of 24 hours 0·1128 gramme; and at the end of 48 hours 0·132 gramme. On digesting 5 grammes of castor-oil seed paste at 38° with 5 c.c. of a 1-per-cent. solution of aspartic acid, 4 c.c. of water and 1 c.c. of toluene, the amount of ricinoleic acid liberated at the end of 19 hours was 2·99 grammes. In a similar experiment with glutamic acid, the amount liberated was

---

* 'American Chemical Journal,' 1900, vol. 24, p. 491.

† 'Zeit. Physiol. Chem.,' 1904, vol. 42, p. 149.

3·024 grammes. On digesting a mixture of 5 grammes of the seed paste, free from husk, with 9 c.c. of water and 1 c.c. of toluene at 38°, the amount of ricinoleic acid liberated after 19 hours was 0·109 gramme; after 116 hours 0·387 gramme; and after 164 hours 0·596 gramme. When chloral hydrate is used as antiseptic, after a time a sudden great increase in the amount of acid liberated is observed; probably this is conditioned by the formation of mineral acid by decomposition of the chloral hydrate.

As acids do not act equally when used in equivalent quantities, although when used in sufficient amount weak acids are as effective as strong acids, it is probable that the strength of the acid is a factor in the action.

All attempts resulted in failure which were made to obtain an extract containing an enzyme, whether from the freshly-ground material directly or after this had been deprived of the fatty matter and whether or no acid were present. It should be mentioned in this connection that Kastle and Loevenhart found that the extract they used lost its activity to a very great extent on mere filtration through paper.

Apparently, acids do not act merely by liberating the enzyme. Several experiments have been made in which the material free from fat was digested, at the temperature at which the hydrolysis is ordinarily effected, with the amount of sulphuric acid in presence of which hydrolysis of fatty oil takes place rapidly; when washed free from acid, the product was incapable of effecting hydrolysis whether used alone or together with fresh acid. Evidently the enzyme had been destroyed.

The Ricinus enzyme has been found to have but little action not only on ethylic butyrate, on acetin and on dimethylic tartrate and racemate, but also on ethylic mandelate, which, according to Dakin, is readily attacked by animal lipase.

It is difficult to resist the impression that the differences observed are not merely consequences of differences in stability of the various ethereal salts but that the Ricinus enzyme is possessed of properties which make it specifically capable of promoting the hydrolysis of glycerides of the higher fatty acids. And in view of recent observations on the action of so-called co-ferments, the part which acids play in promoting hydrolysis is specially interesting.

INDEX TO VOL. LXXVI. (B)

Address at Anniversary, November 30, 1904 (Huggins), 1.
Adie (J. R.) and Alcock (A.) On the Occurrence of *Anopheles* (*Myzomyia*) *Listoni* in Calcutta, 319.
Adrenalin, synthesis of substance allied to (Dakin), 491 ; —— physiological activity of substances related to (Dakin), 498.
Alcock (A.) See Adie and Alcock.
Anopheles Listoni in Calcutta (Adie and Alcock), 319.
Apospory, cytology of (Digby), 463.
Arber (E. A. N.) On some New Species of Lagenostoma, a Type of Pteridospermous Seed from the Coal Measures, 245.
Armstrong (E. F.) Studies on Enzyme Action. VII.—The Synthetic Action of Acids contrasted with that of Enzymes. Synthesis of Maltose and Isomaltose, 592 ; —— VIII.—The Mechanism of Fermentation, 600.
Armstrong (H. E.) Studies on Enzyme Action.—Lipase, 606.
Assheton (R.) The Morphology of the Ungulate Placenta, particularly the Development of that Organ in the Sheep, 393.
Assimilation and respiration, vegetable (Blackman and Matthaei), 402.
Atmospheric carbon dioxide, new method for determining (Brown and Escombe), 112.
Auld (S. J. M.) See Henry and Auld.

Barratt (J. O. W.) The Phagocytosis of Red Blood-cells, 524.
Bastian (H. C.) On the Occurrence of Certain Ciliated Infusoria within the Eggs of a Rotifer, considered from the Point of View of Heterogenesis, 385.
Bateson (W.) and Gregory (R. P.) On the Inheritance of Heterostylism in Primula, 581.
Bikhaconitine, pharmacology of (Cash and Dunstan), 468.
Blackman (F. F.) and Matthaei (G. L. C.) Experimental Researches in Vegetable Assimilation and Respiration. IV.—A Quantitative Study of Carbon-Dioxide Assimilation and Leaf-Temperature in Natural Illumination, 402.
Brown (H. T.) and Escombe (F.) On the Variations in the Amount of Carbon Dioxide in the Air of Kew during the Years 1898–1891, 118 ; —— On a New Method for the Determination of Atmospheric Carbon Dioxide, based on the Rate of its Absorption by a Free Surface of a Solution of Caustic Alkali, 112 ; —— Researches on some of the Physiological Processes of Green Leaves, with special Reference to the Interchange of Energy between the Leaf and its Surroundings, 29 ; —— and Wilson (W. E.) On the Thermal Emissivity of a Green Leaf in Still and Moving Air, 122.
Burch (G. J.) On Colour-Vision by very Weak Light, 199.

Cancer, absence or diminution of hydrochloric acid in stomach, wherever growth situated (Moore), 138 ; —— resemblances between "Plimmer's Bodies" and normal constituents of reproductive cells (Farmer, Moore, and Walker), 230.

Carbon dioxide, absorption by caustic alkali, and determination of in air (Brown an Escombe), 112 ; —— variations in amount of at Kew (Brown and Escombe), 118 ; —— assimilation by plants (Blackman and Matthaei), 402 ; —— effect on geotropism in *Pisum sativum* (Drabble and Lake), 351.

Cash (J. T.) and Dunstan (W. R.) The Pharmacology of Indaconitine and Bikhaconitine, 468.

Cell, dual force of dividing. Spindle figure illustrated by magnetic force-chains (Hartog), 548.

Cephalodiscus nigrescens, new species from Antarctic (Lankester), 400.

Cerebellum, relation to decerebrate rigidity (Thiele), 360.

Chitin in carapace of *Pterygotus osiliensis* (Rosenheim), 398.

Coal measures, type of pteridospermous seed from (Arber), 245.

Cobra-venom, influence on proteid metabolism (Scott), 166.

Colour-vision, trichromic (Edridge-Green), 194 ; —— by very weak light (Burch), 199.

Craw (J. A.) On the Physical Chemistry of the Toxin-Antitoxin Reaction : with special Reference to the Neutralisation of Lysin by Antilysin, 179.

Crustacea, colour physiology of higher (Keeble and Gamble), 198.

Dakin (H. D.) The Synthesis of a Substance allied to Adrenalin, 491 ; —— On the Physiological Activity of Substances indirectly related to Adrenalin, 498.

Dale (E.) Further Experiments and Histological Investigations on Intumescences, with some Observations on Nuclear Division in Pathological Tissues, 587.

Dean (G.) An Experimental Enquiry into the Nature of the Substance in Serum which influences Phagocytosis, 506.

Digby (L.) On the Cytology of Apogamy and Apospory. II.—Preliminary Note on Apospory, 463.

Drabble (E.) and Lake (H.) On the Effect of Carbon Dioxide on Geotropic Curvature of the Roots of *Pisum sativum*, L., 351.

Dunstan (W. R.) See Cash and Dunstan.

Edkins (J. S.) On the Chemical Mechanism of Gastric Secretion, 376.

Edridge-Green (F. W.) Two Cases of Trichromic Vision, 194.

Embryo, early, nutrition of (Emrys-Roberts), 164.

Emrys-Roberts (E.) A Preliminary Note upon the Question of the Nutrition of the Early Embryo, with Special Reference to the Guinea-pig and Man, 164.

Emulsin, probable existence in yeast (Henry and Auld), 568.

Enzyme action, studies on (Armstrong), 592, 600, 606.

Erysiphe graminis, endophytic adaptation of (Salmon), 366.

Escombe (F.) See Brown (H. T.) and Escombe.

Farmer (J. B.), Moore (J. E. S.), and Walker (C. E.) On the Resemblances existing between the "Plimmer's Bodies" of Malignant Growths and certain Normal Constituents of Reproductive Cells of Animals, 230.

Fermentation, mechanism of (Armstrong), 600.

Fever, Mediterranean, relation to goats (Zammit), 377 ; (Horrocks), 378.

Foraminifera, relation in size between parent and offspring (Lister), 298.

Gamble (F. W.) See Keeble and Gamble.

Gastric contents, absence of hydrochloric acid in malignant disease of organs other than stomach (Moore), 138.

Gastric secretion, chemical mechanism of (Edkins), 376.

Geotropic curvature in *Pisum sativum*, effect of carbon dioxide on (Drabble and Lake), 351.
Glossina palpalis, anatomy of (Minchin), 531.
Gregory (R. P.) See Bateson and Gregory.

Hartog (M.) The Dual Force of the Dividing Cell. Part I.—The Achromatic Spindle Figure illustrated by Magnetic Chains of Force, 548.
Heape (Walter) Ovulation and Degeneration of Ova in the Rabbit, 260.
Henry (T. A.) and Auld (S. J. M.) On the Probable Existence of Emulsin in Yeast, 568.
Heterogenesis—ciliated infusoria occurring in eggs of rotifer (Bastian), 385.
Heterostylism in Primula, inheritance of (Bateson and Gregory), 581.
Horrocks (Major W. H.) Preliminary Note on Goats as a Means of Propagation of Mediterranean Fever, 378.
Huggins (Sir Wm.) Address delivered at Anniversary Meeting, November 30, 1904, 1.

Indaconitine, pharmacology of (Cash and Dunstan), 468.
Infusoria, occurrence of ciliated, in eggs of rotifer (Bastian), 385.
Innervation, reciprocal, of antagonistic muscles (Sherrington), 160, 269.
Intumescences, experiments and histological investigations on, 587.

Jolly (W. A.) See Marshall and Jolly.

Keeble (F.) and Gamble (F. W.) The Colour-Physiology of the Higher Crustacea.—Part III, 198.
Kidston (R.) Preliminary Note on the Occurrence of Microsporangia in Organic Connection with the Foliage of Lyginodendron, 358.

Lagenostoma, new species of (Arber), 245.
Lake (H.) See Drabble and Lake.
Lankester (E. R.) On a new Species of Cephalodiscus (*C. nigrescens*) from the Antarctic Ocean, 400.
Larmor (J.) Note on the Mechanics of the Ascent of Sap in Trees, 460.
Leaf, energetics of—thermal relation to surroundings, and absorption of solar radiation (Brown and Escombe), 29 ; —— thermal emissivity of in still and moving air (Brown and Wilson), 122.
Leaves in varied illumination, temperature of (Blackman and Matthaei), 402 ; —— physiological processes—assimilation, photosynthesis (Brown and Escombe), 29.
Lipase (Armstrong), 606.
Lister (J. J.) On the Dimorphism of the English Species of Nummulites, and the Size of the Megalosphere in relation to that of the Microspheric and Megalospheric Tests in this Genus, 298.
Lyginodendron, microsporangia of (Kidston), 358.
Lysin, neutralisation by antilysin (Craw), 179.

Macallum (A. B.) On the Nature of the Silver Reaction in Animal and Vegetable Tissues, 217.
Macdonald (J. S.) The Structure and Function of Nerve-Fibres.—Preliminary Communication, 322.
Malta Fever—relation of goats to (Zammit), 377 ; —— (Horrocks), 378.

Marshall (F. H. A.) and Jolly (W. A.) Contributions to the Physiology of Mammalian Reproduction. Part I.—The Œstrous Cycle in the Dog. Part. II.—The Ovary as an Organ of Internal Secretion, 395.
Matthaei (G. L. C.) See Blackman and Matthaei.
Minchin (E. A.) Report on the Anatomy of the Tsetse-fly (*Glossina palpalis*), 531.
Moore (B.) On the Absence or marked Diminution of Free Hydrochloric Acid in the Gastric Contents, in Malignant Disease of Organs other than the Stomach, 138.
Moore (J. E. S.) See Farmer, Moore and Walker.
Mott (F. W.) Observations on the Brains of Men and Animals infected with various Forms of Trypanosomes. Preliminary Note, 235.
Murray (G.) On a New Rhabdosphere, 243.
Muscles, reciprocal innervation of antagonistic (Sherrington), 160.

Nerve fibres, structure and function of (Macdonald), 322.
Nuclear division in pathological tissues (Dale), 587.
Nummulites, dimorphism of English species of (Lister), 298.

Osmotic theory of ascent of sap (Larmor), 460.
Ovary, as an organ of internal secretion (Marshall and Jolly), 395.
Ovulation and degeneration of ova in rabbit (Heape), 260.

Perrin (W. S.) A Preliminary Communication on the Life-history of *Trypanosoma Balbianii*, 368.
Phagocytosis, nature of substance in serum which influences (Dean), 506; —— of red blood-cells (Barratt), 524.
Placenta, morphology of ungulate (Assheton), 393.
"Plimmer's Bodies" of malignant growths, resemblances to normal constituents of reproductive animal cells (Farmer, Moore, and Walker), 230.
Primula, inheritance of heterostylism in (Bateson and Gregory), 581.
Pterygotus osiliensis, chitin in carapace of (Rosenheim), 398.

Reproduction, physiology of mammalian (Marshall and Jolly), 395.
Respiration and assimilation, vegetable (Blackman and Matthaei), 402.
Rhabdosphere, new species of (Murray), 243.
Rosenheim (O.) Chitin in the Carapace of *Pterygotus osiliensis* from the Silurian Rocks of Oesel, 398.

Salmon (E. S.) On Endophytic Adaptation shown by *Erysiphe graminis* DC. under Cultural Conditions, 366.
Sap in trees, mechanics of ascent of (Larmor), 460.
Scott (James) The Influence of Cobra Venom on the Proteid Metabolism, 166.
Sherrington (C. S.) On Reciprocal Innervation of Antagonistic Muscles.—Seventh Note, 160; Eighth Note, 269.
Silver reaction in animal and vegetable tissues, nature of (Macallum), 217.
Sleeping sickness, changes in lymphatic glands and central nervous system (Mott), 235.
Spinal cord, relationship with optic thalamus and Deiter's nucleus (Thiele), 360.
Spirillum Theileri, transmission and inoculability of (Theiler), 504.

Theiler (A.) Transmission and Inoculability of *Spirillum Theileri* (Laveran), 504.
Thiele (F. H.) On the Efferent Relationship of the Optic Thalamus and Deiter's Nucleus to the Spinal Cord, with Special Reference to the Cerebellar Influx Theory and the Genesis of Decerebrate Rigidity, 360.

Thomas (H. W.) The Experimental Treatment of Trypanosomiasis in Animals, 589.
Toxin-antitoxin reaction, physical chemistry of (Craw), 179.
Trypanosoma Balbianii, life history of (Perrin), 368.
Trypanosoma-infected brains, observations on (Mott), 235.
Trypanosomiasis in animals, experimental treatment of (Thomas), 589.
Tsetse-fly, report on anatomy of (Minchin), 531.

Vision, two cases of trichromic (Edridge-Green), 194 ; —— colour-vision by weak light (Burch), 199.

Walker (C. E.) See Farmer, Moore and Walker.
Wilson (W. E.) See Brown (H. T.) and Wilson.

Yeast, probable existence of emulsin in (Henry and Auld), 568.

Zammit (T.) A Preliminary Note on the Susceptibility of Goats to Malta Fever, 377.

END OF THE SEVENTY-SIXTH VOLUME (SERIES B).

Harrison and Sons, Printers in Ordinary to His Majesty, St. Martin's Lane.

PROCEEDINGS

OF

THE ROYAL SOCIETY.

Series B. Vol. 76. **No. B 507.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

Address delivered by the President, SIR WILLIAM HUGGINS, K.C.B., O.M., F.R.S., at the Anniversary Meeting on November 30th, 1904 . . . 1

Researches on some of the Physiological Processes of Green Leaves, with special Reference to the Interchange of Energy between the Leaf and its Surroundings. By HORACE T. BROWN, LL.D., F.R.S., and F. ESCOMBE . . . 29

On a New Method for the Determination of Atmospheric Carbon Dioxide, based on the Rate of its Absorption by a Free Surface of a Solution of Caustic Alkali. By HORACE T. BROWN, F.R.S., and F. ESCOMBE . . . 112

On the Variations in the Amount of Carbon Dioxide in the Air of Kew during the years 1898-1901. By HORACE T. BROWN, F.R.S., and F. ESCOMBE . . . 118

On the Thermal Emissivity of a Green Leaf in Still and Moving Air. By HORACE T. BROWN, LL.D., F.R.S., and W. E. WILSON, D.Sc., F.R.S. . . . 122

Smithsonian Institution
MAY 9 1905
The Library

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Four Shillings.

No. B 507.
April, 1905.

April 22, 1905.

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Offices and Library will be closed for the Easter vacation from Thursday, April 20th, until Saturday, April 29th, both days included.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent by post to every Fellow of the Society who resides within the limits of the Postal Union. The 'Philosophical Transactions' are delivered only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

PROCEEDINGS

OF

THE ROYAL SOCIETY.

Series B. Vol. 76. **No. B 508.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Absence or Marked Diminution of Free Hydrochloric Acid in the Gastric Contents, in Malignant Disease of Organs other than the Stomach. By BENJAMIN MOORE, M.A., D.Sc., Johnston Professor of Bio-Chemistry in the University of Liverpool 138

On Reciprocal Innervation of Antagonistic Muscles.—Seventh Note. By C. S. SHERRINGTON, F.R.S. 160

A Preliminary Note upon the Question of the Nutrition of the Early Embryo, with Special Reference to the Guinea-pig and Man. By E. EMRYS-ROBERTS, M.B. (Liverpool), Ethel Boyce Research Fellow in Gynæcological Pathology in the University of Liverpool . . . 164

The Influence of Cobra-venom on the Proteid Metabolism. By JAMES SCOTT, M.D., C.M., B.Sc. Edin. 166

On the Physical Chemistry of the Toxin-Antitoxin Reaction: with special reference to the Neutralisation of Lysin by Antilysin. By J. A. CRAW, Research Student, Lister Institute of Preventive Medicine 179

Two Cases of Trichromic Vision. By F. W. EDRIDGE-GREEN, M.D., F.R.C.S. 194

The Colour-Physiology of the Higher Crustacea, Part III. By FREDERICK KEEBLE, M.A., University College, Reading, and F. W. GAMBLE, D.Sc., Zoological Department, Victoria University, Manchester. (Abstract.) . 198

On Colour-Vision by Very Weak Light. By GEORGE J. BURCH, M.A., D.Sc. Oxon.; F.R.S.; Lecturer in Physics, University College, Reading 199

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Two Shillings and Sixpence.

No. B 508.

May 24, 1905.

nian Institution
JUN 5 1905
ce Library

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent by post to every Fellow of the Society who resides within the limits of the Postal Union. The 'Philosophical Transactions' are delivered only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 76. **No. B 509.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Nature of the Silver Reaction in Animal and Vegetable Tissues. By A. B. MACALLUM, M.A., M.B., Ph.D., Professor of Physiology in the University of Toronto 217

On the Resemblances existing between the "Plimmer's Bodies" of Malignant Growths, and Certain Normal Constituents of Reproductive Cells of Animals. By J. BRETLAND FARMER, F.R.S., J. E. S. MOORE, and C. E. WALKER. 230

Observations on the Brains of Men and Animals infected with Various Forms of Trypanosomes. Preliminary Note. By F. W. MOTT, M.D., F.R.S. 235

On a New Rhabdosphere. By GEORGE MURRAY, F.R.S. . . . 243

On some New Species of Lagenostoma, a Type of Pteridospermous Seed from the Coal Measures. By E. A. NEWELL ARBER, M.A., F.L.S., F.G.S., Trinity College, Cambridge, University Demonstrator in Palæobotany. (Plates 1 and 2) 245

Ovulation and Degeneration of Ova in the Rabbit. By WALTER HEAPE, M.A., Trinity College, Cambridge 260

On Reciprocal Innervation of Antagonistic Muscles.—Eighth Note. By C. S. SHERRINGTON, D.Sc., F.R.S. 269

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings and Sixpence.

No. B 509.

June 28, 1905.

Smithsonian Institution,
JUL 10 1905
Office Library.

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent by post to every Fellow of the Society who resides within the limits of the Postal Union. The 'Philosophical Transactions' are delivered only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 76. **No. B 510.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Dimorphism of the English Species of Nummulites, and the Size of the Megalosphere in Relation to that of the Microspheric and Megalospheric Tests in this Genus. By J. J. LISTER, M.A., F.R.S., Fellow of St. John's College and Demonstrator of Comparative Anatomy in the University of Cambridge. (Plates 3—5) **298**

On the Occurrence of Anopheles (Myzomyia) Listoni in Calcutta. By J. R. ADIE, M.B. (Lond.), Major, I.M.S., and A. ALCOCK, M.B., LL.D., F.R.S., Major, I.M.S. **319**

The Structure and Function of Nerve-Fibres.—Preliminary Communication. By J. S. MACDONALD, Professor of Physiology in the University College, Cambridge **322**

On the Effect of Carbon Dioxide on Geotropic Curvature of the Roots of Pisum Sativum L. By ERIC DRABBLE, D.Sc., F.L.S. (Demonstrator in Botany, Royal College of Science), and HILDA LAKE . . . **351**

Preliminary Note on the Occurrence of Microsporangia in Organic Connection with the Foliage of Lyginodendron. By R. KIDSTON, F.R.S.L. and E., F.G.S. (Plate 6) **358**

On the Efferent Relationship of the Optic Thalamus and Deiter's Nucleus to the Spinal Cord, with Special Reference to the Cerebellar Influx Theory (Hughlings Jackson) and the Genesis of Decerebrate Rigidity (Sherrington). By F. H. THIELE, M.D., B.Sc., M.R.C.P., Pathologist to University College Hospital **360**

On Endophytic Adaptation shown by Erysiphe Graminis DC. under Cultural Conditions. By ERNEST S. SALMON, F.L.S. (Abstract) **366**

A Preliminary Communication on the Life History of Trypanosoma Balbianii. By W. S. PERRIN, B.A., Shuttleworth Research Student of Gonville and Caius College, Cambridge **368**

For continuation of Contents see 2nd page of Wrapper.

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings and Sixpence.

No. B 510.

August 3, 1905.

Smithsonian Institution,
AUG 12 1905.
Office Library.

CONTENTS (*continued*).

Page

On the Chemical Mechanism of Gastric Secretion. By J. S. EDKINS, M.A., M.B. Cantab., Lecturer on Physiology in the Medical School of St. Bartholomew's Hospital, London 376

A Preliminary Note on the Susceptibility of Goats to Malta Fever. By Dr. T. ZAMMIT, Member of the Mediterranean Fever Commission . 377

Preliminary Note on Goats as a Means of Propagation of Mediterranean Fever. By Major W. H. HORROCKS, R.A.M.C., Member of the Mediterranean Fever Commission 378

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

During August and September the Offices and Library of the Society will be closed at 4, on Saturdays at 1.

The Library will be closed for cleaning from August 8 to August 19.

No books can be borrowed during August, and all books out are ordered by Council to be returned by August 1.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent by post to every Fellow of the Society who resides within the limits of the Postal Union. The 'Philosophical Transactions' are delivered only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 76. **No. B 511.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Occurrence of Certain Ciliated Infusoria within the Eggs of a Rotifer, considered from the Point of View of Heterogenesis. By H. CHARLTON BASTIAN, M.A., M.D., F.R.S. (Abstract.) (Plate 7) 385

The Morphology of the Ungulate Placenta, particularly the Development of that Organ in the Sheep, and Notes upon the Placenta of the Elephant and Hyrax. By RICHARD ASSHETON, M.A., Lecturer on Biology in the Medical School of Guy's Hospital, University of London. (Abstract) 393

Contributions to the Physiology of Mammalian Reproduction. Part I.—The Œstrous Cycle in the Dog. Part II.—The Ovary as an Organ of Internal Secretion. By FRANCIS H. A. MARSHALL, Carnegie Fellow in the University of Edinburgh; and WILLIAM A. JOLLY, Assistant to the Professor of Physiology in the University of Edinburgh. (Abstract) . 395

Chitin in the Carapace of Pterygotus osiliensis, from the Silurian Rocks of Oesel. By OTTO ROSENHEIM, Ph.D. 398

On a New Species of Cephalodiscus (C. nigrescens) from the Antarctic Ocean. By E. RAY LANKESTER, M.A., D.Sc., LL.D., F.R.S., Director of the Natural History Departments of the British Museum. (Plate 8) . . 400

Experimental Researches in Vegetable Assimilation and Respiration. IV.—A Quantitative Study of Carbon-Dioxide Assimilation and Leaf-Temperature in Natural Illumination. By F. FROST BLACKMAN, D.Sc., Fellow of St. John's College, Reader in Botany in the University of Cambridge, and GABRIELLE L. C. MATTHAEI, Fellow of Newnham College . . 402

Note on the Mechanics of the Ascent of Sap in Trees. By Professor J. LARMOR, Sec. R.S. 460

hsonian Institution,
SEP 15 1905
Library

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Four Shillings.

No. B 511.

September 2, 1905.

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

During August and September the Offices and Library of the Society are closed at 4, on Saturdays at 1.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent by post to every Fellow of the Society who resides within the limits of the Postal Union. The 'Philosophical Transactions' are delivered only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 76. No. B 512.

BIOLOGICAL SCIENCES.

CONTENTS.

Page

On the Cytology of Apogamy and Apospory.—II. Preliminary Note on Apospory. By Miss L. DIGBY 463

The Pharmacology of Indaconitine and Bikhaconitine. By J. THEODORE CASH, M.D., F.R.S., and WYNDHAM R. DUNSTAN, M.A., LL.D., F.R.S. 468

The Synthesis of a Substance allied to Adrenalin. By H. D. DAKIN . . 491

On the Physiological Activity of Substances Indirectly Related to Adrenalin. By H. D. DAKIN 498

Transmission and Inoculability of Spirillum Theileri (Laveran). By Dr. A. THEILER 504

An Experimental Enquiry into the Nature of the Substance in Serum which influences Phagocytosis. By GEORGE DEAN, M.A., C.M., M.B., Bacteriologist-in-Charge of the Serum Department of the Lister Institute of Preventive Medicine, London 506

The Phagocytosis of Red Blood-Cells. By J. O. WAKELIN BARRATT, M.D., B.Sc. Lond., British Medical Association Research Student . . 524

Report on the Anatomy of the Tsetse-fly (Glossina palpalis). By E. A. MINCHIN, M.A., Professor of Zoology and Comparative Anatomy in University College, London 531

Smithsonian Institution, OCT 19 1905 Office Library.

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Two Shillings and Sixpence.

No. B 512.

October 7, 1905.

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent in the ordinary course by post to every Fellow of the Society who resides within the limits of the Postal Union, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane; these will be bound in volumes, in cloth, for 2s. 6d., or the cases for binding may be purchased, price 1s. 6d.

The 'Philosophical Transactions' are delivered, in volumes, in cloth covers, only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free, as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 76. **No. B 513.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Dual Force of the Dividing Cell. Part I: The Achromatic Spindle Figure Illustrated by Magnetic Chains of Force. By MARCUS HARTOG, M.A., D.Sc., F.R.U.I. (Plates 9—11) 548

On the Probable Existence of Emulsin in Yeast. By THOMAS ANDERSON HENRY, D.Sc. (Lond.), Principal Assistant in the Scientific and Technical Department of the Imperial Institute, and S. J. M. AULD, Ph.D. (Würzburg), Assistant in the Scientific and Technical Department of the Imperial Institute 568

On the Inheritance of Heterostylism in Primula. By W. BATESON, M.A., F.R.S., and R. P. GREGORY, M.A. 581

Further Experiments and Histological Investigations on Intumescences, with some Observations on Nuclear Division in Pathological Tissues. By Miss ELIZABETH DALE. (Abstract) 587

The Experimental Treatment of Trypanosomiasis in Animals. By H. WOLFERSTAN THOMAS, M.D., C.M. (McGill), (J. H. Todd Memorial Fellow in Tropical Medicine), Liverpool School of Tropical Medicine, Johnston Tropical Laboratory, University, Liverpool . . 589

Studies on Enzyme Action. VII.—The Synthetic Action of Acids contrasted with that of Enzymes. Synthesis of Maltose and Isomaltose. By EDWARD FRANKLAND ARMSTRONG, Ph.D., D.Sc., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College 592

Studies on Enzyme Action. VIII.—The Mechanism of Fermentation. By EDWARD FRANKLAND ARMSTRONG, Ph.D., D.Sc., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College 600

Studies on Enzyme Action.—Lipase. By HENRY E. ARMSTRONG, F.R.S. 606

Index 609

Title, Contents, &c.

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings.

No. B 513.

November 9, 1905.

Smithsonian Institution
NOV 29 1905
Office Library

NOTICE TO AUTHORS.

Authors of Papers are urgently requested to send in all Drawings, Diagrams, or other Illustrations, in a state suitable for direct photographic reproduction. They should be drawn on a large scale, in Indian ink, on a smooth white surface, with lettering adapted to a reduction in scale. Where illustrations are numerous, much time would be saved if authors would indicate in advance those considered essential to the understanding of their papers.

(When the MS. is typewritten the copy should be carefully corrected by the author before it is presented.)

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent in the ordinary course by post to every Fellow of the Society who resides within the limits of the Postal Union, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane; these will be bound in volumes, in cloth, for 2s. 6d., or the cases for binding may be purchased, price 1s. 6d.

The 'Philosophical Transactions' are delivered, in volumes, in cloth covers, only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The separate Papers forming the 'Philosophical Transactions' will be sent post free, as issued, to those Fellows who prefer to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

PHILOSOPHICAL TRANSACTIONS

1904—1905.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 203 (1904). Pp. 422. With 2 Plates. Price £1 1s. 6d.
Vol. 204 (1905). Pp. 500. With 4 Plates. Price £1 9s. 0d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904). Pp. 390. With 23 Plates. Price £1 16s. 0d.
Vol. 197 (1905). Pp. 408. With 22 Plates. Price £1 13s. 6d.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 390. THE HALOGEN HYDRIDES AS CONDUCTING SOLVENTS. By B. D. STEELE, D.Sc., D. McINTOSH, M.A., D.Sc., and E. H. ARCHIBALD, M.A., Ph.D. Part I.—THE VAPOUR PRESSURES, DENSITIES, SURFACE ENERGIES AND VISCOSITIES OF THE PURE SOLVENTS. By D. McINTOSH and B. D. STEELE. Part II.—THE CONDUCTIVITY AND MOLECULAR WEIGHTS OF DISSOLVED SUBSTANCES. By D. McINTOSH and E. H. ARCHIBALD. Part III.—THE TRANSPORT NUMBERS OF CERTAIN DISSOLVED SUBSTANCES. By B. D. STEELE. Part IV.—THE ABNORMAL VARIATION OF MOLECULAR CONDUCTIVITY, ETC. By B. D. STEELE, D. McINTOSH, and E. H. ARCHIBALD. Pp. 69. Price 4s.

A. 391. THE ATOMIC WEIGHT OF CHLORINE: AN ATTEMPT TO DETERMINE THE EQUIVALENT OF CHLORINE BY DIRECT BURNING WITH HYDROGEN. By HAROLD B. DIXON, M.A., F.R.S. (late Fellow of Balliol College, Oxford), Professor of Chemistry, and E. C. EDGAR, B.Sc., Dalton Scholar of the University of Manchester. Pp. 32. Price 1s. 6d.

A. 392. RESEARCHES ON EXPLOSIVES—Part III. By Sir ANDREW NOBLE, Bart., K.C.B., F.R.S., F.R.A.S. Pp. 36; 13 Plates. Price 1s.

A. 393. COLOURS IN METAL GLASSES, IN METALLIC FILMS, AND IN METALLIC SOLUTIONS.—II. By J. C. MAXWELL GARNETT. Pp. 52. Price 3s.

A. 394. ON THE INTENSITY AND DIRECTION OF THE FORCE OF GRAVITY IN INDIA. By Lieut.-Colonel S. G. BURRARD, R.E., F.R.S. Pp. 29; 7 Plates. Price 2s. 6d.

A. 395. ON THE REFRACTIVE INDEX OF GASEOUS FLUORINE. By C. CUTHBERTSON and E. B. R. PRIDEAUX, M.A., B.Sc. Pp. 13. Price 1s.

B. 240. ON THE STRUCTURE AND AFFINITIES OF FOSSIL PLANTS FROM THE PALÆOZOIC ROCKS. V. ON A NEW TYPE OF SPHENOPHYLLACEOUS CONE (*SPHENOPHYLLUM FERTILE*) FROM THE LOWER COAL-MEASURES. By D. H. SCOTT, M.A., Ph.D., F.R.S., Honorary Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew. Pp. 23; 3 Plates. Price 2s. 6d.

B. 241. THE ASCENT OF WATER IN TREES. By ALFRED J. EWART, D.Sc., Ph.D., F.L.S., Lecturer on Botany in the Birmingham University, and in the Birmingham Municipal Technical School. Pp. 45. Price 2s.

B. 242. ON ENDOPHYTIC ADAPTATION SHOWN BY *ERYSIPHE GRAMINIS* DC. UNDER CULTURAL CONDITIONS. By ERNEST S. SALMON, F.L.S. Pp. 11; 1 Plate. Price 1s.

B. 243. CONTRIBUTIONS TO THE PHYSIOLOGY OF MAMMALIAN REPRODUCTION. Part I.—THE ŒSTROUS CYCLE IN THE DOG. Part II.—THE OVARY AS AN ORGAN OF INTERNAL SECRETION. By FRANCIS H. A. MARSHALL, Carnegie Fellow in the University of Edinburgh; and WILLIAM A. JOLLY, Assistant to the Professor of Physiology in the University of Edinburgh. Pp. 43; 2 Plates. Price 5s.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s.*

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s.* 6*d.*

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s.*; to Fellows, 20*s.*

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. HERDMAN, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s.*; to Fellows, 15*s.* Part II., 1904, Pp. 300, with 37 Plates, Price 20*s.*; to Fellows, 15*s.*; Part III, 1905, Pp. 384, with 34 Plates, Price 20*s.*; to Fellows, 15*s.*

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. ALDO CASTELLANI, Lieut.-Col. BRUCE, and Dr. DAVID NABARRO.	1903.	Price 7*s.* 6*d.*
,, II.	By Drs. C. CHRISTY, A. CASTELLANI, and G. C. LOW.	1903.	Price 7*s.* 6*d.*
,, III.	By Dr. C. CHRISTY and F. V. THEOBALD.	1903.	Price 6*s.*
,, IV.	By Lieut.-Col. BRUCE, Dr. NABARRO, and Capt. GREIG.	1903.	Price 6*s.* 6*d.*
,, V.	By Dr. NABARRO and Capt. GREIG.	1905.	Price 3*s.*
,, VI.	By Capt. GREIG and Lieut. GRAY (Published by H.M. Stationery Office).	1905.	Price 4*s.* 6*d.*

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. DANIELS, STEPHENS, CHRISTOPHERS, and THEOBALD.	1900.	Price 2*s.* 6*d.*
2nd ,,	By Messrs. CHRISTOPHERS and STEPHENS.	1900.	Price 1*s.* 6*d.*
3rd ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1900.	Price 2*s.*
4th ,,	By S. R. CHRISTOPHERS.	1901.	Price 3*s.* 6*d.*
5th ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1901.	Price 2*s.* 6*d.*
6th ,,	By Messrs. STEPHENS and CHRISTOPHERS.	1902.	Price 1*s.*
7th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Prof. RAY LANKESTER.	1902.	Price 3*s.*
8th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Capt. JAMES.	1903.	Price 2*s.*

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report 1.	By W. BATESON, F.R.S., and Miss SAUNDERS. Pp. 160.	1902.	Price 10*s.*
Report 2.	By W. BATESON, F.R.S., Miss SAUNDERS, R. C. PUNNETT, M.A., and C. C. HURST, F.L.S. Pp. 154.	1905.	Price 7*s.* 6*d.*

Contents of PROCEEDINGS, No. A 512. September, 1905.

Price 2s. 6d.

Page

THE ELASTIC PROPERTIES OF STEEL AT HIGH TEMPERATURES. By BERTRAM HOPKINSON, M.A., M.I.C.E., Professor of Mechanism and Applied Mechanics in the University of Cambridge, and F. ROGERS, B.A. (Cantab.), M.Sc., M.Eng. 419

ON THE REFRACTIVE INDEX OF GASEOUS FLUORINE. By C. CUTHBERTSON and E. B. R. PRIDEAUX, M.A., B.Sc. (Abstract) 426

THE ABSORPTION SPECTRUM AND FLUORESCENCE OF MERCURY VAPOUR. By W. N. HARTLEY, D.Sc., F.R.S. 428

THE FORMATION OF ICE AND THE GRAINED STRUCTURE OF GLACIERS. By G. QUINCKE, For. Mem. R.S., Professor of Physics in the University of Heidelberg .. 431

ON THE INFLUENCE OF COLLISIONS AND OF THE MOTION OF MOLECULES IN THE LINE OF SIGHT, UPON THE CONSTITUTION OF A SPECTRUM LINE. By LORD RAYLEIGH, O.M., F.R.S... 440

AN EXPERIMENT WITH THE BALANCE TO FIND IF CHANGE OF TEMPERATURE HAS ANY EFFECT UPON WEIGHT. By J. H. POYNTING, Sc.D., F.R.S., and PERCY PHILLIPS, M.Sc. 445

A NEW FORMATION OF DIAMOND. By Sir WILLIAM CROOKES, Hon. D.Sc. (Oxford and Dubl.), F.R.S... 458

THE INFLUENCE OF PHASE CHANGES ON THE TENACITY OF DUCTILE METALS AT THE ORDINARY TEMPERATURE AND AT THE BOILING POINT OF LIQUID AIR. By G. T. BEILBY and H. N. BEILBY, B.Sc. (Plate 5) 462

THE REFRACTIVE INDICES OF SULPHURIC ACID AT DIFFERENT CONCENTRATIONS. By V. H. VELEY, F.R.S., and J. J. MANLEY, M.A. 469

ON THE SPECTRUM OF THE SPONTANEOUS LUMINOUS RADIATION OF RADIUM. PART III.—RADIATION IN HYDROGEN. By Sir WILLIAM HUGGINS, K.C.B., O.M., D.C.L., Pres. R.S., and LADY HUGGINS, Hon. Mem. R.A.S. 488

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

PHILOSOPHICAL TRANSACTIONS

1904–1905.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 203 (1904).	Pp. 422.	With 2 Plates.	Price £1 1s. 6d.
Vol. 204 (1905).	Pp. 500.	With 4 Plates.	Price £1 9s. 0d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904).	Pp. 390.	With 23 Plates.	Price £1 16s. 0d.
Vol. 197 (1905).	Pp. 408.	With 22 Plates.	Price £1 13s. 6d.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 388. MEMOIR ON THE THEORY OF THE PARTITIONS OF NUMBERS—PART III. By P. A MACMAHON, Major R.A., Sc.D., F.R.S. Pp. 23. Price 1s.

A. 389. ATMOSPHERIC ELECTRICITY IN HIGH LATITUDES. By GEORGE C. SIMPSON, B.Sc. (1851 Exhibition Scholar of the University of Manchester). Pp. 37. Price 3s.

A. 390. THE HALOGEN HYDRIDES AS CONDUCTING SOLVENTS. By B. D. STEELE, D.Sc., D. McINTOSH, M.A., D.Sc., and E. H. ARCHIBALD, M.A., Ph.D. Part I.—THE VAPOUR PRESSURES, DENSITIES, SURFACE ENERGIES AND VISCOSITIES OF THE PURE SOLVENTS. By D. McINTOSH and B. D. STEELE. Part II.—THE CONDUCTIVITY AND MOLECULAR WEIGHTS OF DISSOLVED SUBSTANCES. By D. McINTOSH and E. H. ARCHIBALD. Part III.—THE TRANSPORT NUMBERS OF CERTAIN DISSOLVED SUBSTANCES. By B. D. STEELE. Part IV.—THE ABNORMAL VARIATION OF MOLECULAR CONDUCTIVITY, ETC. By B. D. STEELE, D. McINTOSH, and E. H. ARCHIBALD. Pp. 69. Price 4s.

A. 391. THE ATOMIC WEIGHT OF CHLORINE: AN ATTEMPT TO DETERMINE THE EQUIVALENT OF CHLORINE BY DIRECT BURNING WITH HYDROGEN. By HAROLD B. DIXON, M.A., F.R.S. (late Fellow of Balliol College, Oxford), Professor of Chemistry, and E. C. EDGAR, B.Sc., Dalton Scholar of the University of Manchester. Pp. 32. Price 1s. 6d.

A. 392. RESEARCHES ON EXPLOSIVES—Part III. By Sir ANDREW NOBLE, Bart., K.C.B., F.R.S., F.R.A.S. Pp. 36; 13 Plates. Price 1s.

B. 239. THE COLOUR-PHYSIOLOGY OF HIGHER CRUSTACEA. Part III. By FREDERICK KEEBLE, M.A., University College, Reading, and F. W. GAMBLE, D.Sc., Zoological Department, Victoria University of Manchester. Pp. 16; 2 Plates. Price 1s. 6d.

B. 240. ON THE STRUCTURE AND AFFINITIES OF FOSSIL PLANTS FROM THE PALÆOZOIC ROCKS. V. ON A NEW TYPE OF SPHENOPHYLLACEOUS CONE (*SPHENOPHYLLUM FERTILE*) FROM THE LOWER COAL-MEASURES. By D. H. SCOTT, M.A., Ph.D., F.R.S., Honorary Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew. Pp. 23; 3 Plates. Price 2s. 6d.

B. 241. THE ASCENT OF WATER IN TREES. By ALFRED J. EWART, D.Sc., Ph.D., F.L.S., Lecturer on Botany in the Birmingham University, and in the Birmingham Municipal Technical School. Pp. 45. Price 2s.

B. 242. ON ENDOPHYTIC ADAPTATION SHOWN BY *ERYSIPHE GRAMINIS* DC. UNDER CULTURAL CONDITIONS. By ERNEST S. SALMON, F.L.S. Pp. 11; 1 Plate. Price 1s.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s*.

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s*. 6*d*.

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s*.; to Fellows, 20*s*.

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. HERDMAN, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s*.; to Fellows, 15*s*. Part II., 1904, Pp. 300, with 37 Plates, Price 20*s*.; to Fellows, 15*s*.; Part III, 1905, Pp. 384, with 34 Plates, Price 20*s*.; to Fellows, 15*s*.

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. ALDO CASTELLANI, Lieut.-Col. BRUCE, and Dr. DAVID NABARRO.	1903.	Price 7*s*. 6*d*.
,, II.	By Drs. C. CHRISTY, A. CASTELLANI, and G. C. LOW.	1903.	Price 7*s*. 6*d*.
,, III.	By Dr. C. CHRISTY and F. V. THEOBALD.	1903.	Price 6*s*.
,, IV.	By Lieut.-Col. BRUCE, Dr. NABARRO, and Capt. GREIG.	1903.	Price 6*s*. 6*d*.
,, V.	By Dr. NABARRO and Capt. GREIG.	1905.	Price 3*s*.
,, VI.	By Capt. GREIG and Lieut. GRAY (Published by H.M. Stationery Office).	1905.	Price 4*s*. 6*d*.

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. DANIELS, STEPHENS, CHRISTOPHERS, and THEOBALD.	1900.	Price 2*s*. 6*d*.
2nd ,,	By Messrs. CHRISTOPHERS and STEPHENS.	1900.	Price 1*s*. 6*d*.
3rd ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1900.	Price 2*s*.
4th ,,	By S. R. CHRISTOPHERS.	1901.	Price 3*s*. 6*d*.
5th ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1901.	Price 2*s*. 6*d*.
6th ,,	By Messrs. STEPHENS and CHRISTOPHERS.	1902.	Price 1*s*.
7th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Prof. RAY LANKESTER.	1902.	Price 3*s*.
8th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Capt. JAMES.	1903.	Price 2*s*.

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report 1.	By W. BATESON, F.R.S., and Miss SAUNDERS. Pp. 160.	1902.	Price 10*s*.
Report 2.	By W. BATESON, F.R.S., Miss SAUNDERS, R. C. PUNNETT, M.A., and C. C. HURST, F.L.S. Pp. 154.	1905.	Price 7*s*. 6*d*.

Contents of PROCEEDINGS, No. A 512. September, 1905.

Price 2s. 6d.

Page

THE ELASTIC PROPERTIES OF STEEL AT HIGH TEMPERATURES. By BERTRAM HOPKINSON, M.A., M.I.C.E., Professor of Mechanism and Applied Mechanics in the University of Cambridge, and F. ROGERS, B.A. (Cantab.), M.Sc., M.Eng. 419

ON THE REFRACTIVE INDEX OF GASEOUS FLUORINE. By C. CUTHBERTSON and E. B. R. PRIDEAUX, M.A., B.Sc. (Abstract) 426

THE ABSORPTION SPECTRUM AND FLUORESCENCE OF MERCURY VAPOUR. By W. N. HARTLEY, D.Sc., F.R.S. 428

THE FORMATION OF ICE AND THE GRAINED STRUCTURE OF GLACIERS. By G. QUINCKE, For. Mem. R.S., Professor of Physics in the University of Heidelberg .. 431

ON THE INFLUENCE OF COLLISIONS AND OF THE MOTION OF MOLECULES IN THE LINE OF SIGHT, UPON THE CONSTITUTION OF A SPECTRUM LINE. By LORD RAYLEIGH, O.M., F.R.S... 440

AN EXPERIMENT WITH THE BALANCE TO FIND IF CHANGE OF TEMPERATURE HAS ANY EFFECT UPON WEIGHT. By J. H. POYNTING, Sc.D., F.R.S., and PERCY PHILLIPS, M.Sc. 445

A NEW FORMATION OF DIAMOND. By Sir WILLIAM CROOKES, Hon. D.Sc. (Oxford and Dubl.), F.R.S... 458

THE INFLUENCE OF PHASE CHANGES ON THE TENACITY OF DUCTILE METALS AT THE ORDINARY TEMPERATURE AND AT THE BOILING POINT OF LIQUID AIR. By G. T. BEILBY and H. N. BEILBY, B.Sc. (Plate 5) 462

THE REFRACTIVE INDICES OF SULPHURIC ACID AT DIFFERENT CONCENTRATIONS. By V. H. VELEY, F.R.S., and J. J. MANLEY, M.A. 469

ON THE SPECTRUM OF THE SPONTANEOUS LUMINOUS RADIATION OF RADIUM. PART III.—RADIATION IN HYDROGEN. By Sir WILLIAM HUGGINS, K.C.B., O.M., D.C.L., Pres. R.S., and LADY HUGGINS, Hon. Mem. R.A.S. 488

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

PHILOSOPHICAL TRANSACTIONS

1904–1905.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 203 (1904). Pp. 422. With 2 Plates. Price £1 1s. 6d.
Vol. 204 (1905). Pp. 500. With 4 Plates. Price £1 9s. 0d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904). Pp. 390. With 23 Plates. Price £1 16s. 0d.
Vol. 197 (1905). Pp. 408. With 22 Plates. Price £1 13s. 6d.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 387. ON THE NORMAL SERIES SATISFYING LINEAR DIFFERENTIAL EQUATIONS. By E. CUNNINGHAM, B.A., Fellow of St. John's College, Cambridge. Pp. 35. Price 1s. 6d.

A. 388. MEMOIR ON THE THEORY OF THE PARTITIONS OF NUMBERS—PART III. By P. A. MACMAHON, Major R.A., Sc.D., F.R.S. Pp. 23. Price 1s.

A. 389. ATMOSPHERIC ELECTRICITY IN HIGH LATITUDES. By GEORGE C. SIMPSON, B.Sc. (1851 Exhibition Scholar of the University of Manchester). Pp. 37. Price 3s.

A. 390. THE HALOGEN HYDRIDES AS CONDUCTING SOLVENTS. By B. D. STEELE, D.Sc., D. McINTOSH, M.A., D.Sc., and E. H. ARCHIBALD, M.A., Ph.D. Part I.—THE VAPOUR PRESSURES, DENSITIES, SURFACE ENERGIES AND VISCOSITIES OF THE PURE SOLVENTS. By D. McINTOSH and B. D. STEELE. Part II.—THE CONDUCTIVITY AND MOLECULAR WEIGHTS OF DISSOLVED SUBSTANCES. By D. McINTOSH and E. H. ARCHIBALD. Part III.—THE TRANSPORT NUMBERS OF CERTAIN DISSOLVED SUBSTANCES. By B. D. STEELE. Part IV.—THE ABNORMAL VARIATION OF MOLECULAR CONDUCTIVITY, ETC. By B. D. STEELE, D. McINTOSH, and E. H. ARCHIBALD. Pp. 69. Price 4s.

A. 391. THE ATOMIC WEIGHT OF CHLORINE: AN ATTEMPT TO DETERMINE THE EQUIVALENT OF CHLORINE BY DIRECT BURNING WITH HYDROGEN. By HAROLD B. DIXON, M.A., F.R.S. (late Fellow of Balliol College, Oxford), Professor of Chemistry, and E. C. EDGAR, B.Sc., Dalton Scholar of the University of Manchester. Pp. 32. Price 1s. 6d.

B. 239. THE COLOUR-PHYSIOLOGY OF HIGHER CRUSTACEA. Part III. By FREDERICK KEEBLE, M.A., University College, Reading, and F. W. GAMBLE, D.Sc., Zoological Department, Victoria University of Manchester. Pp. 16; 2 Plates. Price 1s. 6d.

B. 240. ON THE STRUCTURE AND AFFINITIES OF FOSSIL PLANTS FROM THE PALÆOZOIC ROCKS. V. ON A NEW TYPE OF SPHENOPHYLLACEOUS CONE (*SPHENOPHYLLUM FERTILE*) FROM THE LOWER COAL-MEASURES. By D. H. SCOTT, M.A., Ph.D., F.R.S., Honorary Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew. Pp. 23; 3 Plates. Price 2s. 6d.

B. 241. THE ASCENT OF WATER IN TREES. By ALFRED J. EWART, D.Sc., Ph.D., F.L.S., Lecturer on Botany in the Birmingham University, and in the Birmingham Municipal Technical School. Pp. 45. Price 2s.

B. 242. ON ENDOPHYTIC ADAPTATION SHOWN BY *ERYSIPHE GRAMINIS* DC. UNDER CULTURAL CONDITIONS. By ERNEST S. SALMON, F.L.S. Pp. 11; 1 Plate. Price 1s.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s.*

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s.* 6*d.*

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s.*; to Fellows, 20*s.*

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. HERDMAN, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s.*; to Fellows, 15*s.* Part II., 1904, Pp. 300, with 37 Plates, Price 20*s.*; to Fellows, 15*s.*; Part III, 1905, Pp. 384, with 34 Plates, Price 20*s.*; to Fellows, 15*s.*

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. ALDO CASTELLANI, Lieut.-Col. BRUCE, and Dr DAVID NABARRO.	1903.	Price 7*s.* 6*d.*
,, II.	By Drs. C. CHRISTY, A. CASTELLANI, and G. C. LOW.	1903.	Price 7*s.* 6*d.*
,, III.	By Dr. C. CHRISTY and F. V. THEOBALD.	1903.	Price 6*s.*
,, IV.	By Lieut.-Col. BRUCE, Dr. NABARRO, and Capt. GREIG.	1903.	Price 6*s.* 6*d.*
,, V.	By Dr. NABARRO and Capt. GREIG.	1905.	Price 3*s.*

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. DANIELS, STEPHENS, CHRISTOPHERS, and THEOBALD.	1900.	Price 2*s.* 6*d.*
2nd ,,	By Messrs. CHRISTOPHERS and STEPHENS.	1900.	Price 1*s.* 6*d.*
3rd ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1900.	Price 2*s.*
4th ,,	By S. R. CHRISTOPHERS.	1901.	Price 3*s.* 6*d.*
5th ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1901.	Price 2*s.* 6*d.*
6th ,,	By Messrs. STEPHENS and CHRISTOPHERS.	1902.	Price 1*s.*
7th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Prof. RAY LANKESTER.	1902.	Price 3*s.*
8th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Capt. JAMES.	1903.	Price 2*s.*

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report 1.	By W. BATESON, F.R.S., and Miss SAUNDERS. Pp. 160.	1902.	Price 10*s.*
Report 2.	By W. BATESON, F.R.S., Miss SAUNDERS, R. C. PUNNETT, M.A., and C. C. HURST, F.L.S. Pp. 154.	1905.	Price 7*s.* 6*d.*

Contents of PROCEEDINGS, No. A 511. August, 1905.

Price 2s. 6d.

Page

EXPLOSIONS OF MIXTURES OF COAL-GAS AND AIR IN A CLOSED VESSEL. By LEONARD BAIRSTOW, A.R.C.Sc., Wh. Sch., and A. D. ALEXANDER, A.R.C.Sc., Wh. Sch. (Abstract) 340

THE IMPROVED ELECTRIC MICROMETER. By P. E. SHAW, B.A., D.Sc. 350

THE AMPLITUDE OF THE MINIMUM AUDIBLE IMPULSIVE SOUND. By P. E. SHAW, B.A., D.Sc. 360

NOTE ON DR. H. A. WILSON'S MEMOIR "ON THE ELECTRIC EFFECT OF ROTATING A DIELECTRIC IN A MAGNETIC FIELD." By S. J. BARNETT, Palo Alto, California, U.S.A. 367

COLOURS IN METAL GLASSES, IN METALLIC FILMS AND IN METALLIC SOLUTIONS.—II. By J. C. MAXWELL GARNETT. (Abstract) 370

THE EVOLUTION OF THE SPECTRUM OF A STAR DURING ITS GROWTH FROM A NEBULA. By W. E. WILSON, D.Sc., F.R.S. 374

RESEARCHES ON EXPLOSIVES.—PART III. By Sir ANDREW NOBLE, Bart., K.C.B., F.R.S., D.Sc., etc. (Abstract) 381

THE ANALOGY BETWEEN LESAGE'S THEORY OF GRAVITATION AND THE REPULSION OF LIGHT. By G. H. DARWIN, F.R.S., Plumian Professor and Fellow of Trinity College, in the University of Cambridge 387

ON THE PHOSPHORESCENT SPECTRA OF $S\delta$ AND EUROPIUM. By Sir WILLIAM CROOKES, D.Sc., F.R.S. 411

RECORDS OF DIFFERENCE OF TEMPERATURE BETWEEN MCGILL COLLEGE OBSERVATORY AND THE TOP OF MOUNT ROYAL, MONTREAL. By Professor C. McLEOD, F.R.S. Can. 415

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

PHILOSOPHICAL TRANSACTIONS

1904—1905.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 203 (1904).	Pp. 422.	With 2 Plates.	Price £1 1s. 6d.
Vol. 204 (1905).	Pp. 500.	With 4 Plates.	Price £1 9s. 0d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904).	Pp. 390.	With 23 Plates.	Price £1 16s. 0d.
Vol. 197 (1905).	Pp. 408.	With 22 Plates.	Price £1 13s. 6d.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 384. POLARISED RÖNTGEN RADIATION. By CHARLES G. BARKLA, D.Sc. (Liverpool), M.Sc. (Vict.), B.A. (Cantab.), King's College, Cambridge; Oliver Lodge Fellow, University of Liverpool. Pp. 13. Price 1s.

A. 385. ON THE CONVERGENCE OF INFINITE SERIES OF ANALYTIC FUNCTIONS. By H. A. WEBB, B.A., Fellow of Trinity College, Cambridge. Pp. 17. Price 1s.

A. 386. TITLE, CONTENTS, INDEX, &c., Vol. 204. Price 6d.

A. 387. ON THE NORMAL SERIES SATISFYING LINEAR DIFFERENTIAL EQUATIONS. By E. CUNNINGHAM, B.A., Fellow of St. John's College, Cambridge. Pp. 35. Price 1s. 6d.

A. 388. MEMOIR ON THE THEORY OF THE PARTITIONS OF NUMBERS—PART III. By P. A. MACMAHON, Major R.A., Sc.D., F.R.S. Pp. 23. Price 1s.

A. 389. ATMOSPHERIC ELECTRICITY IN HIGH LATITUDES. By GEORGE C. SIMPSON, B.Sc. (1851 Exhibition Scholar of the University of Manchester). Pp. 37. Price 3s.

B. 237. A CONTRIBUTION TO THE STUDY OF THE ACTION OF INDIAN COBRA VENOM. By Captain R. H. ELLIOT, M.B., B.S.Lond., F.R.C.S.Eng., D.P.H.Camb., etc. Pp. 46. Price 4s.

B. 238. TITLE, CONTENTS, INDEX, &c., Vol. 197. Price 6d.

B. 239. THE COLOUR-PHYSIOLOGY OF HIGHER CRUSTACEA. Part III. By FREDERICK KEEBLE, M.A., University College, Reading, and F. W. GAMBLE, D.Sc., Zoological Department, Victoria University of Manchester. Pp. 16; 2 Plates. Price 1s. 6d.

B. 240. ON THE STRUCTURE AND AFFINITIES OF FOSSIL PLANTS FROM THE PALÆOZOIC ROCKS. V. ON A NEW TYPE OF SPHENOPHYLLACEOUS CONE (*SPHENOPHYLLUM FERTILE*) FROM THE LOWER COAL-MEASURES. By D. H. SCOTT, M.A., Ph.D., F.R.S., Honorary Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew. Pp. 23; 3 Plates. Price 2s. 6d.

B. 241. THE ASCENT OF WATER IN TREES. By ALFRED J. EWART, D.Sc., Ph.D., F.L.S., Lecturer on Botany in the Birmingham University, and in the Birmingham Municipal Technical School. Pp. 45. Price 2s.

B. 242. ON ENDOPHYTIC ADAPTATION SHOWN BY *ERYSIPHE GRAMINIS* DC. UNDER CULTURAL CONDITIONS. By ERNEST S. SALMON, F.L.S. Pp. 11; 1 Plate. Price 1s.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s*.

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s*. 6*d*.

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s*.; to Fellows, 20*s*.

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. Herdman, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s*.; to Fellows, 15*s*. Part II., 1904, Pp. 300, with 37 Plates, Price 20*s*.; to Fellows, 15*s*.; Part III, 1905, Pp. 384, with 34 Plates, Price 20*s*.; to Fellows, 15*s*.

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. Aldo Castellani, Lieut.-Col. Bruce, and Dr David Nabarro.	1903.	Price 7*s*. 6*d*.
,, II.	By Drs. C. Christy, A. Castellani, and G. C. Low.	1903.	Price 7*s*. 6*d*.
,, III.	By Dr. C. Christy and F. V. Theobald.	1903.	Price 6*s*.
,, IV.	By Lieut.-Col. Bruce, Dr. Nabarro, and Capt. Greig.	1903.	Price 6*s*. 6*d*.

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. Daniels, Stephens, Christophers, and Theobald.	1900.	Price 2*s*. 6*d*.
2nd ,,	By Messrs. Christophers and Stephens.	1900.	Price 1*s*. 6*d*.
3rd ,,	By Messrs. Stephens, Christophers, and Daniels.	1900.	Price 2*s*.
4th ,,	By S. R. Christophers.	1901.	Price 3*s*. 6*d*.
5th ,,	By Messrs. Stephens, Christophers, and Daniels.	1901.	Price 2*s*. 6*d*.
6th ,,	By Messrs. Stephens and Christophers.	1902.	Price 1*s*.
7th ,,	By Messrs. Stephens and Christophers and Prof. Ray Lankester.	1902.	Price 3*s*.
8th ,,	By Messrs. Stephens and Christophers and Capt. James.	1903.	Price 2*s*.

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report 1.	By W. Bateson, F.R.S., and Miss Saunders. Pp. 160.	1902.	Price 10*s*.
Report 2.	By W. Bateson, F.R.S., Miss Saunders, R. C. Punnett, M.A., and C. C. Hurst, F.L.S. Pp. 154.	1905.	Price 7*s*. 6*d*.

Contents of PROCEEDINGS, No. A 511. August, 1905.

Price 2s. 6d.

Page

EXPLOSIONS OF MIXTURES OF COAL-GAS AND AIR IN A CLOSED VESSEL. By Leonard Bairstow, A.R.C.Sc., Wh. Sch., and A. D. Alexander, A.R.C.Sc., Wh. Sch. (Abstract) 340

THE IMPROVED ELECTRIC MICROMETER. By P. E. Shaw, B.A., D.Sc. 350

THE AMPLITUDE OF THE MINIMUM AUDIBLE IMPULSIVE SOUND. By P. E. Shaw, B.A., D.Sc. 360

NOTE ON DR. H. A. WILSON'S MEMOIR "ON THE ELECTRIC EFFECT OF ROTATING A DIELECTRIC IN A MAGNETIC FIELD." By S. J. Barnett, Palo Alto, California, U.S.A. 367

COLOURS IN METAL GLASSES, IN METALLIC FILMS AND IN METALLIC SOLUTIONS.—II. By J. C. Maxwell Garnett. (Abstract) 370

THE EVOLUTION OF THE SPECTRUM OF A STAR DURING ITS GROWTH FROM A NEBULA. By W. E. Wilson, D.Sc., F.R.S. 374

RESEARCHES ON EXPLOSIVES.—PART III. By Sir Andrew Noble, Bart., K.C.B., F.R.S., D.Sc., etc. (Abstract) 381

THE ANALOGY BETWEEN LESAGE'S THEORY OF GRAVITATION AND THE REPULSION OF LIGHT. By G. H. Darwin, F.R.S., Plumian Professor and Fellow of Trinity College, in the University of Cambridge 387

ON THE PHOSPHORESCENT SPECTRA OF Sδ AND EUROPIUM. By Sir William Crookes, D.Sc., F.R.S. 411

RECORDS OF DIFFERENCE OF TEMPERATURE BETWEEN MCGILL COLLEGE OBSERVATORY, AND THE TOP OF MOUNT ROYAL, MONTREAL. By Professor C. McLeod, F.R.S. Can. 415

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

PHILOSOPHICAL TRANSACTIONS

1904—1905.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 203 (1904).	Pp. 422.	With 2 Plates.	Price £1 1s. 6d.
Vol. 204 (1905).	Pp. 500.	With 4 Plates.	Price £1 9s. 0d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904).	Pp. 390.	With 23 Plates.	Price £1 16s. 0d.
Vol. 197 (1905).	Pp. 408.	With 22 Plates.	Price £1 13s. 6d.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 381. AN ANALYSIS OF THE RESULTS FROM THE FALMOUTH MAGNETOGRAPHS ON "QUIET" DAYS DURING THE TWELVE YEARS 1891 TO 1902. By CHARLES CHREE, Sc.D., LL.D., F.R.S. Pp. 34. Price 2s.

A. 382. ON THE MODULUS OF TORSIONAL RIGIDITY OF QUARTZ FIBRES AND ITS TEMPERATURE COEFFICIENT. By FRANK HORTON, D.Sc., B.A., St. John's College, Cambridge, Mackinnon Student of the Royal Society. Pp. 25. Price 1s.

A. 383. EFFECTS OF TEMPERATURE AND PRESSURE ON THE THERMAL CONDUCTIVITIES OF SOLIDS.—PART I. THE EFFECT OF TEMPERATURE ON THE THERMAL CONDUCTIVITIES OF SOME ELECTRICAL INSULATORS. By CHARLES H. LEES, D.Sc. Pp. 34. Price 2s.

A. 384. POLARISED RÖNTGEN RADIATION. By CHARLES G. BARKLA, D.Sc. (Liverpool), M.Sc. (Vict.), B.A. (Cantab.), King's College, Cambridge; Oliver Lodge Fellow, University of Liverpool. Pp. 13. Price 1s.

A. 385. ON THE CONVERGENCE OF INFINITE SERIES OF ANALYTIC FUNCTIONS. By H. A. WEBB, B.A., Fellow of Trinity College, Cambridge. Pp. 17. Price 1s.

A. 386. TITLE, CONTENTS, INDEX, &c., Vol. 204. Price 6d.

B. 236. FURTHER NOTE ON THE REMAINS OF *ELEPHAS CYPRIOTES* FROM A CAVE-DEPOSIT IN CYPRUS. By DOROTHEA M. A. BATE. Pp. 14; 2 Plates. Price 2s.

B. 237. A CONTRIBUTION TO THE STUDY OF THE ACTION OF INDIAN COBRA VENOM. By Captain R. H. ELLIOT, M.B., B.S.Lond., F.R.C.S.Eng. D.P.H.Camb., etc. Pp. 46. Price 4s.

B. 238. TITLE, CONTENTS, INDEX, &c., Vol. 197. Price 6d.

B. 239. THE COLOUR-PHYSIOLOGY OF HIGHER CRUSTACEA. Part III. By FREDERICK KEEBLE, M.A., University College, Reading, and F. W. GAMBLE, D.Sc., Zoological Department, Victoria University of Manchester. Pp. 16; 2 Plates. Price 1s. 6d.

B. 240. ON THE STRUCTURE AND AFFINITIES OF FOSSIL PLANTS FROM THE PALÆOZOIC ROCKS. V. ON A NEW TYPE OF SPHENOPHYLLACEOUS CONE (*SPHENOPHYLLUM FERTILE*) FROM THE LOWER COAL-MEASURES. By D. H. SCOTT, M.A., Ph.D., F.R.S., Honorary Keeper of the Jodrell Laboratory, Royal Botanic Gardens, Kew. Pp. 23; 3 Plates. Price 2s. 6d.

B. 241. THE ASCENT OF WATER IN TREES. By ALFRED J. EWART, D.Sc., Ph.D., F.L.S., Lecturer on Botany in the Birmingham University, and in the Birmingham Municipal Technical School. Pp. 45. Price 2s.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s.*

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s.* 6*d.*

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s.*; to Fellows, 20*s.*

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. HERDMAN, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s.*; to Fellows, 15*s.* Part II., 1904, Pp. 300, with 37 Plates, Price 20*s.*; to Fellows, 15*s.*; Part III, 1905, Pp. 384, with 34 Plates, Price 20*s.*; to Fellows, 15*s.*

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. ALDO CASTELLANI, Lieut.-Col. BRUCE, and Dr DAVID NABARRO.	1903.	Price 7*s.* 6*d.*
,, II.	By Drs. C. CHRISTY, A. CASTELLANI, and G. C. LOW.	1903.	Price 7*s.* 6*d.*
,, III.	By Dr. C. CHRISTY and F. V. THEOBALD.	1903.	Price 6*s.*
,, IV.	By Lieut.-Col. BRUCE, Dr. NABARRO, and Capt. GREIG.	1903.	Price 6*s.* 6*d.*

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. DANIELS, STEPHENS, CHRISTOPHERS, and THEOBALD.	1900.	Price 2*s.* 6*d.*
2nd ,,	By Messrs. CHRISTOPHERS and STEPHENS.	1900.	Price 1*s.* 6*d.*
3rd ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1900.	Price 2*s.*
4th ,,	By S. R. CHRISTOPHERS.	1901.	Price 3*s.* 6*d.*
5th ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1901.	Price 2*s.* 6*d.*
6th ,,	By Messrs. STEPHENS and CHRISTOPHERS.	1902.	Price 1*s.*
7th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Prof. RAY LANKESTER.	1902.	Price 3*s.*
8th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Capt. JAMES.	1903.	Price 2*s.*

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report 1.	By W. BATESON, F.R.S., and Miss SAUNDERS. Pp. 160.	1902.	Price 10*s.*
Report 2.	By W. BATESON, F.R.S., Miss SAUNDERS, R. C. PUNNETT, M.A., and C. C. HURST, F.L.S. Pp. 154.	1905.	Price 7*s.* 6*d.*

Contents of PROCEEDINGS, No. A 509. June, 1905.

Price 2s. 6d.

Page

REPORT ON AN AREA OF LOCAL MAGNETIC DISTURBANCE IN EAST LOCH ROAG, LEWES, HEBRIDES. By Captain ARTHUR MOSTYN FIELD, Royal Navy 181

THE DETERMINATION OF THE SPECIFIC HEAT OF SUPERHEATED STEAM BY THROTTLING AND OTHER EXPERIMENTS. By A. H. PEAKE, M.A., A.I.E.E. .. 185

ON THE DISTRIBUTION OF VELOCITY IN A VISCOUS FLUID OVER THE CROSS-SECTION OF A PIPE, AND ON THE ACTION AT THE CRITICAL VELOCITY. By JOHN MORROW, M.Sc. (Vict.), M.Eng. (Liverpool), Lecturer in Engineering, University College, Bristol 205

THE THEORY OF PHOTOGRAPHIC PROCESSES, PART II: ON THE CHEMICAL DYNAMICS OF DEVELOPMENT, INCLUDING THE MICROSCOPY OF THE IMAGE. By S. E. SHEPPARD, B.Sc., and C. E. K. MEES, B.Sc. (Plate 4) 217

ON A NEW TYPE OF ELECTRIC FURNACE, WITH A REDETERMINATION OF THE MELTING-POINT OF PLATINUM. By J. A. HARKER, D.Sc., Joule Student of the Royal Society, Assistant at the National Physical Laboratory, Teddington 235

THE ATOMIC WEIGHT OF CHLORINE: AN ATTEMPT TO DETERMINE THE EQUIVALENT OF CHLORINE BY DIRECT BURNING WITH HYDROGEN. By HAROLD B. DIXON, M.A., F.R.S. (late Fellow of Balliol College, Oxford), Professor of Chemistry, and E. C. EDGAR, B.Sc., Dalton Scholar of the University of Manchester. (Abstract) 250

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

PHILOSOPHICAL TRANSACTIONS

1903–1904.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 202 (1903). Pp. 554. With 13 Plates. Price £1 13s. 0d.
Vol. 203 (1904). Pp. 422. With 2 Plates. Price £1 1s. 6d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904). Pp. 390. With 23 Plates. Price £1 16s. 0d.
Vol. 197 (1905). Pp. 408. With 22 Plates. Price £1 13s. 6d.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 378. THE ELECTRICAL CONDUCTIVITY AND OTHER PROPERTIES OF SODIUM HYDROXIDE IN AQUEOUS SOLUTION AS ELUCIDATING THE MECHANISM OF CONDUCTION. By WILLIAM ROBERT BOUSFIELD, M.A., K.C., M.P., and THOMAS MARTIN LOWRY, D.Sc. Pp. 70. Price 5s.

A. 379. OF THE REFRACTIVE INDICES OF THE ELEMENTS. By CLIVE CUTHBERTSON. Pp. 27. Price 1s.

A. 380. ON THE COMPRESSIBILITY OF GASES BETWEEN ONE ATMOSPHERE AND HALF AN ATMOSPHERE OF PRESSURE. By LORD RAYLEIGH, O.M., F.R.S. Pp. 22. Price 1s.

A. 381. AN ANALYSIS OF THE RESULTS FROM THE FALMOUTH MAGNETOGRAPHS ON "QUIET" DAYS DURING THE TWELVE YEARS 1891 TO 1902. By CHARLES CHREE, Sc.D., LL.D., F.R.S. Pp. 34. Price 2s.

A. 382. ON THE MODULUS OF TORSIONAL RIGIDITY OF QUARTZ FIBRES AND ITS TEMPERATURE COEFFICIENT. By FRANK HORTON, D.Sc., B.A., St. John's College, Cambridge, Mackinnon Student of the Royal Society. Pp. 25. Price 1s.

B. 234. THE FOSSIL FLORA OF THE CULM MEASURES OF NORTH-WEST DEVON AND THE PALÆOBOTANICAL EVIDENCE WITH REGARD TO THE AGE OF THE BEDS. By E. A. NEWELL ARBER, M.A., F.L.S., F.G.S. Pp. 35; 2 Plates. Price 2s. 6d.

B. 235. ON THE ACTION OF THE VENOM OF *BUNGARUS CŒRULEUS* (THE COMMON KRAIT). By Major R. H. ELLIOT, I.M.S.; W. C. SILLAR, M.B., B.Sc., and GEORGE S. CARMICHAEL, M.D. Pp. 19. Price 1s. 6d.

B. 236. FURTHER NOTE ON THE REMAINS OF *ELEPHAS CYPRIOTES* FROM A CAVE-DEPOSIT IN CYPRUS. By DOROTHEA M. A. BATE. Pp. 14; 2 Plates. Price 2s.

B. 237. A CONTRIBUTION TO THE STUDY OF THE ACTION OF INDIAN COBRA VENOM. By Captain R. H. ELLIOT, M.B., B.S.Lond., F.R.C.S.Eng. D.P.H.Camb., etc. Pp. 46. Price 4s.

B. 238. TITLE, CONTENTS, INDEX, &c., Vol. 197. Price 6d.

B. 239. THE COLOUR-PHYSIOLOGY OF HIGHER CRUSTACEA. Part III. By FREDERICK KEEBLE, M.A., University College, Reading, and F. W. GAMBLE, D.Sc., Zoological Department, Victoria University of Manchester. Pp. 16; 2 Plates. Price 1s. 6d.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s*.

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s*. 6*d*.

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s*.; to Fellows, 20*s*.

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. Herdman, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s*.; to Fellows, 15*s*. Part II., 1904, Pp. 300, with 37 Plates, Price 20*s*.; to Fellows, 15*s*.

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. Aldo Castellani, Lieut.-Col. Bruce, and Dr David Nabarro.	1903.	Price 7*s*. 6*d*.
„ II.	By Drs. C. Christy, A. Castellani, and G. C. Low.	1903.	Price 7*s*. 6*d*.
„ III.	By Dr. C. Christy and F. V. Theobald.	1903.	Price 6*s*.
„ IV.	By Lieut.-Col. Bruce, Dr. Nabarro, and Capt. Greig.	1903.	Price 6*s*. 6*d*.

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. Daniels, Stephens, Christophers, and Theobald.	1900.	Price 2*s*. 6*d*.
2nd „	By Messrs. Christophers and Stephens.	1900.	Price 1*s*. 6*d*.
3rd „	By Messrs. Stephens, Christophers, and Daniels.	1900.	Price 2*s*.
4th „	By S. R. Christophers.	1901.	Price 3*s*. 6*d*.
5th „	By Messrs. Stephens, Christophers, and Daniels.	1901.	Price 2*s*. 6*d*.
6th „	By Messrs. Stephens and Christophers.	1902.	Price 1*s*.
7th „	By Messrs. Stephens and Christophers and Prof. Ray Lankester.	1902.	Price 3*s*.
8th „	By Messrs. Stephens and Christophers and Capt. James.	1903.	Price 2*s*.

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report I. By W. Bateson, F.R.S., and Miss Saunders. Pp. 160.	1902.	Price 10*s*.
Report 2. By W. Bateson, F.R.S., Miss Saunders, R. C. Punnett, M.A., and C. C. Hurst, F.L.S. Pp. 154.	1905.	Price 7*s*. 6*d*.

Contents of PROCEEDINGS, No. A 508. May, 1905.

Price 3s. od.

Page

ON THE RADIO-ACTIVE MINERALS. By the Hon. R. J. Strutt, Fellow of Trinity College, Cambridge .. 88

THE RATE OF TRANSMISSION OF THE GUATEMALA EARTHQUAKE, APRIL 19, 1902. By R. D. Oldham .. 102

A DETERMINATION OF THE AMOUNTS OF NEON AND HELIUM IN ATMOSPHERIC AIR. By Sir William Ramsay, K.C.B., F.R.S. .. 111

A NEW RADIO-ACTIVE ELEMENT, WHICH EVOLVES THORIUM EMANATION. PRELIMINARY COMMUNICATION. By O. Hahn, Ph.D. .. 115

ON THE SPECTRUM OF SILICON; WITH A NOTE ON THE SPECTRUM OF FLUORINE. By Joseph Lunt, B.Sc., F.I.C. (Plate 2.) .. 118

THE BASIC GAMMA-FUNCTION AND THE ELLIPTIC FUNCTIONS. By the Rev. F. H. Jackson, R.N. .. 127

FURTHER RESEARCHES ON THE TEMPERATURE CLASSIFICATION OF STARS. No. 2. By Sir Norman Lockyer, K.C.B., Hon. LL.D. (Glasgow), Hon. Sc.D. (Camb.), F.R.S. (Plate 3) .. 145

ON SOME CONTINUOUS OBSERVATIONS OF THE RATE OF DISSIPATION OF ELECTRIC CHARGES IN THE OPEN AIR. By C. Coleridge Farr, D.Sc. .. 152

ATMOSPHERIC ELECTRICITY IN HIGH LATITUDES. By George C. Simpson, B.Sc. (Abstract.) .. 160

NOTE ON FLUORESCENCE AND ABSORPTION. By John Butler Burke, M.A. .. 165

THE DIRECT SYNTHESIS OF AMMONIA. By Edgar Philip Perman, D.Sc., Assistant Professor of Chemistry at University College, Cardiff .. 167

DETERMINATION OF VAPOUR-PRESSURE BY AIR-BUBBLING. By Edgar Philip Perman and John Hughes Davies .. 174

ON THE OBSERVATIONS OF STARS MADE IN SOME BRITISH STONE CIRCLES.—PRELIMINARY NOTE. By Sir Norman Lockyer, K.C.B., F.R.S., LL.D., Sc.D. .. 177

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

PHILOSOPHICAL TRANSACTIONS

1903—1904.

SERIES A. CONTAINING PAPERS OF A MATHEMATICAL OR PHYSICAL CHARACTER.

Vol. 202 (1903). Pp. 554. With 13 Plates. Price £1 13s. 0d.
Vol. 203 (1904). Pp. 422. With 2 Plates. Price £1 1s. 6d.

SERIES B. CONTAINING PAPERS OF A BIOLOGICAL CHARACTER.

Vol. 196 (1904). Pp. 390. With 23 Plates. Price £1 16s. 0d.
Vol. 197 (1905). Pp. 408. With 22 Plates. Price £1 13s. 6d.

RECENT PAPERS IN THE 'PHILOSOPHICAL TRANSACTIONS.'

Sold separately by Dulau & Co., 37, Soho Square, London, W.

A. 374. ON THE ELECTRIC EFFECT OF ROTATING A DIELECTRIC IN A MAGNETIC FIELD. By HAROLD A. WILSON, M.A., D.Sc., Fellow of Trinity College, Cambridge. Pp. 17. Price 1s.

A. 375. ON FLAME SPECTRA. By CHARLES DE WATTEVILLE. Pp. 30; 1 Plate. Price 2s.

A. 376. BAKERIAN LECTURE—THE SUCCESSION OF CHANGES IN RADIO-ACTIVE BODIES. By E. RUTHERFORD, F.R.S., Macdonald Professor of Physics, McGill University, Montreal. Pp. 51. Price 3s.

A. 377. ON THE GENERAL THEORY OF INTEGRATION. By W. H. YOUNG, Sc.D., St. Peter's College, Cambridge. Pp. 32. Price 1s.

A. 378. THE ELECTRICAL CONDUCTIVITY AND OTHER PROPERTIES OF SODIUM HYDROXIDE IN AQUEOUS SOLUTION AS ELUCIDATING THE MECHANISM OF CONDUCTION. By WILLIAM ROBERT BOUSFIELD, M.A., K.C., M.P., and THOMAS MARTIN LOWRY, D.Sc. Pp. 70. Price 5s.

A. 379. OF THE REFRACTIVE INDICES OF THE ELEMENTS. By CLIVE CUTHBERTSON. Pp. 27. Price 1s.

B. 232. CONTRIBUTIONS TO THE STUDY OF THE ACTION OF SEA-SNAKE VENOMS. Part I.—Venoms of *Enhydrina Valakadien* and *Enhydris Curtus*. By Sir THOMAS R. FRASER, M.D., F.R.S., Professor of Materia Medica in the University of Edinburgh, and Major R. H. ELLIOT, I.M.S. Pp. 31. Price 2s.

B. 233. ON THE ACTION OF WOOD ON A PHOTOGRAPHIC PLATE IN THE DARK. By WILLIAM J. RUSSELL, Ph.D., F.R.S. Pp. 9; 8 Plates. Price 2s. 6d.

B. 234. THE FOSSIL FLORA OF THE CULM MEASURES OF NORTH-WEST DEVON AND THE PALÆOBOTANICAL EVIDENCE WITH REGARD TO THE AGE OF THE BEDS. By E. A. NEWELL ARBER, M.A., F.L.S., F.G.S. Pp. 35; 2 Plates. Price 2s. 6d.

B. 235. ON THE ACTION OF THE VENOM OF *BUNGARUS CŒRULEUS* (THE COMMON KRAIT). By Major R. H. ELLIOT, I.M.S.; W. C. SILLAR, M.B., B.Sc., and GEORGE S. CARMICHAEL, M.D. Pp. 19. Price 1s. 6d.

B. 236. FURTHER NOTE ON THE REMAINS OF *ELEPHAS CYPRIOTES* FROM A CAVE-DEPOSIT IN CYPRUS. By DOROTHEA M. A. BATE. Pp. 14; 2 Plates. Price 2s.

B. 237. A CONTRIBUTION TO THE STUDY OF THE ACTION OF INDIAN COBRA VENOM. By Captain R. H. ELLIOT, M.B., B.S.Lond., F.R.C.S.Eng., D.P.H.Camb., etc. Pp. 46. Price 4s.

PUBLISHED BY THE ROYAL SOCIETY.

YEAR-BOOK OF THE ROYAL SOCIETY OF LONDON, 1905. 8vo. Pp. 257. Price 5*s.*

THE RECORD OF THE ROYAL SOCIETY OF LONDON, containing Historical Documents and Full Lists of Fellows. 2nd Edition, 1901. Demy 8vo, cloth. Pp. 427. Price 7*s.* 6*d.*

THE ATOLL OF FUNAFUTI: BORINGS INTO A CORAL REEF AND THE RESULTS. 1903. 4to, cloth boards. Pp. 428, with numerous Plates and Diagrams. Price 30*s.*; to Fellows, 20*s.*

REPORT ON THE PEARL OYSTER FISHERIES OF THE GULF OF MANAAR. By W. A. HERDMAN, F.R.S. With Supplementary Reports by other Naturalists upon the Marine Biology. 4to, cloth boards. Part I., 1903, Pp. 307, with 27 Plates, Price 20*s.*; to Fellows, 15*s.* Part II., 1904, Pp. 300, with 37 Plates, Price 20*s.*; to Fellows, 15*s.*

REPORTS OF THE SLEEPING SICKNESS COMMISSION OF THE ROYAL SOCIETY. Demy 8vo.

No. I.	By Dr. ALDO CASTELLANI, Lieut.-Col. BRUCE, and Dr. DAVID NABARRO.	1903.	Price 7*s.* 6*d.*
,, II.	By Drs. C. CHRISTY, A. CASTELLANI, and G. C. LOW.	1903.	Price 7*s.* 6*d.*
,, III.	By Dr. C. CHRISTY and F. V. THEOBALD.	1903.	Price 6*s.*
,, IV.	By Lieut.-Col. BRUCE, Dr. NABARRO, and Capt. GREIG.	1903.	Price 6*s.* 6*d.*

REPORTS TO THE MALARIA COMMITTEE OF THE ROYAL SOCIETY. 8vo.

1st Series.	By Messrs. DANIELS, STEPHENS, CHRISTOPHERS, and THEOBALD.	1900.	Price 2*s.* 6*d.*
2nd ,,	By Messrs. CHRISTOPHERS and STEPHENS.	1900.	Price 1*s.* 6*d.*
3rd ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1900.	Price 2*s.*
4th ,,	By S. R. CHRISTOPHERS.	1901.	Price 3*s.* 6*d.*
5th ,,	By Messrs. STEPHENS, CHRISTOPHERS, and DANIELS.	1901.	Price 2*s.* 6*d.*
6th ,,	By Messrs. STEPHENS and CHRISTOPHERS.	1902.	Price 1*s.*
7th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Prof. RAY LANKESTER.	1902.	Price 3*s.*
8th ,,	By Messrs. STEPHENS and CHRISTOPHERS and Capt. JAMES.	1903.	Price 2*s.*

REPORTS TO THE EVOLUTION COMMITTEE OF THE ROYAL SOCIETY. Demy 8vo.

Report I.	By W. BATESON, F.R.S., and Miss SAUNDERS. Pp. 160.	1902.	Price 10*s.*
Report 2.	By W. BATESON, F.R.S., Miss SAUNDERS, R. C. PUNNETT, M.A., and C. C. HURST, F.L.S. Pp. 154.	1905.	Price 7*s.* 6*d.*

Contents of PROCEEDINGS, No. A 507. April, 1905.

Price 2s. 6d.

Page

ADDRESS DELIVERED BY THE PRESIDENT, SIR WILLIAM HUGGINS, K.C.B., O.M., F.R.S., AT THE ANNIVERSARY MEETING, NOVEMBER 30TH, 1904 1

THE BORING OF THE SIMPLON TUNNEL, AND THE DISTRIBUTION OF TEMPERATURE THAT WAS ENCOUNTERED. By FRANCIS FOX, M.Inst.C.E. 29

ON A METHOD OF FINDING THE CONDUCTIVITY FOR HEAT. By Prof. C. NIVEN, F.R.S. 34

THEORY OF THE REFLECTION OF LIGHT NEAR THE POLARISING ANGLE. By RICHARD C. MACLAURIN, M.A., LL.D. 49

ON THE RELATION BETWEEN VARIATIONS OF ATMOSPHERIC PRESSURE IN NORTH-EAST AFRICA AND THE NILE FLOOD. By Capt. H. G. LYONS. (Plate I.) .. ..

1823 9b

SOLD BY HARRISON & SONS, 45, ST. MARTIN'S LANE, W.C.

SMITHSONIAN INSTITUTION LIBRARIES
3 9088 01306 4456